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VISCOSITY AS A MEASURE OF HYDRATION CAPACITY OF WHEAT FLOUR AND ITS RELATION TO BAKING STRENGTH

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA

BY

PAUL FRANCIS SHARP, A.B., M.S.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

June 1922

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Paul F. Sharp.

VISCOSITY AS A MEASURE OF HYDRATION CAPACITY OF WHEAT FLOUR AND ITS RELATION TO BAKING STRENGTH

By Paul Francis Sharp with Ross Aiken Gortner

INTRODUCTION

It has long been a matter of common knowledge that flours produced from different wheats vary in their baking qualities. Millers and bakers commonly designate flours of good baking qualities as "strong" and those of inferior baking qualities as "weak." The definition of "strength" found in the literature which is commonly accepted is that formulated by the English workers Humphries and Biffen (1907).

"A strong wheat is one which yields flour capable of making large well-piled loaves; the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily."

Guthrie (1896) makes the following statement, altho his use of the term "strength" is not commonly employed.

"The property possessed by flour of absorbing water, known technically as the strength of the flour, is known to vary considerably in the different samples. In order to produce a dough of a given consistency, flour from different grain takes up quite different proportions of water. This is a factor of the greatest importance to the bread-maker, and consequently to the bread consumer, as well as to the miller and farmer, since upon it depends the volume and lightness of the baked loaf."

In England, Humphries has evolved a method of grading bread in which a numerical value called "bakers' mark" is assigned to the bread. Humphries (1908) states that in fifty or sixty cases he had awarded marks in the usual way and then measured the loaves. He found that the marks indicated the volume within a small percentage of error.

In the United States the volume of the loaf produced by a definite weight of flour when baked under a given set of conditions is usually taken as a measure of strength. Briggs (1913), in a discussion of the baking test, emphasizes the significance of loaf volume and points out that large volume of loaf and good texture are related. Thomas (1917), in addition to showing the relation of volume to strength, also pointed out that there was a correlation of strength with texture of the loaf as well.

As the volume of the loaf enters to a greater or less degree into all these definitions, the attempt was made in this work to use that as the main index of strength.

A certain correlation has been established between the strength of the flour yielded by different wheats and the geographical distribution of the wheats. Thomas (1917) summarized a great amount of data on this point. Soil, length of growing season, and moisture are probably the governing factors as indicated by many workers, notably Shutt (1909), Le Clerc and Leavitt (1910), Thatcher (1913), and Le Clerc and Yoder (1914).

Millers and bakers have long known that the wheats grown in the Northern Great Plains area of North America, that is, the spring wheat sections, produce flours having baking qualities superior to those grown in eastern, southern, and western regions. For this reason the geographical source of the wheat is often taken as an indication of the strength or weakness of the flour that can be milled from it. Owing to the overlapping of the regions in which the different types of wheat are grown, and to local and seasonal variations, the geographical source of the wheat in many instances gives only a rough approximation of the baking strength. The data presented by Thomas (1917) show this very clearly. Furthermore, certain varieties of wheat grown in the same geographical region yield flours of greater strength than others. The work of Biffin (1905-06) (1908-10), Thatcher (1913), Howard (1913) (1914), and others emphasized the importance of this factor, and as a result plant breeders have made a marked advance in increasing the strength of wheat grown in various regions by the introduction and development of new varieties and the elimination of undesirable types.

Baking tests are now used extensively in determining flour strength. Such tests are in many respects purely arbitrary procedures, and standardization of testing methods is to be desired. Various formulas are followed, and various treatments are accorded in the several testing laboratories. The skill of the baker also determines in large measure the characteristics of the test loaf. For these reasons it is impossible to compare the results of tests made in different laboratories unless they are all stated in terms of a common control sample, which is usually not the case.

While the procedure in measuring and scoring the test loaf is likewise variable, the comparative cubical dimensions (volume), and crumb texture of the loaf are commonly regarded as most significant. In estimating the latter the judgment of the operator is called into play, while the former may be measured with more or less precision and

expressed in numerical values. For this reason loaf volume is most frequently employed as a criterion of flour strength.

Even tho an exact and standard method were available for conducting practical baking tests, it would still be necessary to regard the characteristics of the resultant loaves as the function of several variables. Thus the test might indicate any substantial deviation from the standard or type flour, without, of necessity, demonstrating the factor. Numerous workers have investigated almost every known chemical and physical property of flour that could possibly be correlated with strength. As yet all the limiting factors have not been discovered, or their inter-relationship recognized. The investigations reported in this paper constitute an effort to determine the significant properties of wheat gluten which may be correlated with baking strength.

HISTORICAL REVIEW

FLOUR STRENGTH

In the following discussion no attempt is made to review all the contributions to the literature on flour strength, but rather to review the more important factors which have been investigated by various workers in the attempt to throw light on the problem of flour strength.

Crude gluten.—Beccari (1745), professor of medicine, anatomy, and chemistry at the University of Bologna, was apparently the first to report the washing of crude gluten from flour with water. He says that he orally communicated to the Academy, in 1728, the fact that wheat flour can be separated into two parts, one of vegetable, the other of animal character.

Many investigators have attempted to correlate the amount of this crude gluten which a given flour will yield, with its baking strength. The process of determination, as ordinarily carried out, is to weigh out a definite quantity of flour, add enough water to form a dough, allow it to stand for some time, and then wash away the starch with water. The material remaining is called the wet gluten. It may be weighed and the results expressed in percentage of wet gluten on the basis of the original flour. The wet gluten is usually dried and the results are expressed in percentage of dry crude gluten on the basis of the original flour. The percentage of dry matter in the wet gluten is frequently calculated.

Millon (1854), while investigating the composition of wheat, pointed out the relationship between wet and dry gluten, and between dry gluten and the protein content of various flours.

As the different investigators began to use the amount of crude gluten as a measure of the baking properties of flour, they discovered

many sources of error in the determination. Benard and Girardin (1881) found that the length of time the dough stood before washing out the gluten influenced the yield of gluten. Balland (1884) confirmed their results, showing in addition that the yield of gluten, as influenced by the time of standing, passed through a maximum. The time of reaching this maximum was found to vary with different flours. He showed that under the same conditions the percentage yield of gluten increased with the size of the sample of flour taken for the determination, also that between the limits of 40 to 60 grams of water per 100 grams of flour used in making the dough, the yield of gluten was the same altho harder to gather with the larger amounts of water. He found that hard wheat flour lost less gluten by continued washing than did soft wheat flour. Balland (1893) showed that the temperature at which the dough was made, allowed to stand, and washed, made a slight difference in the yield of gluten and that the ease with which it could be gathered and retained in the washing process varied. At a temperature of 2° C., he obtained from a given sample of flour 27 per cent of wet gluten; at 15° C., 27.6 per cent; and at 60° C., 30 per cent. Arpin (1902), reports results for the yield of dry gluten at the various temperatures showing an increase for a given flour. When washed at a temperature of 5° C., the yield of dry gluten was 7.83 per cent; at 15° C., 8.08 per cent; at 25° C., 8.24 per cent; and at 35° C., 8.46 per cent. He also showed that there was an increase in the yield of dry gluten of 4.7 per cent if hard water instead of distilled water was used in washing the gluten. Arpin also pointed out that moist gluten would take up additional water if allowed to stand in water, and that washing the material five minutes longer than the usual time decreased the percentage of both moist and dry gluten.

Balland (1884) (1893), Stein (1904), and others tried the effect of mixing various electrolytes with the dough and then washing out the gluten, and found that some electrolytes had little effect with normal flours and in many cases obtained no gluten.

Lindet and Ammann (1905) studied the effect of admixtures of lower grades of wheat flour on the washing out of the gluten from normal flours and in many cases obtained no gluten.

Fleurent (1905) recommends that distilled water containing 0.1 gram of calcium carbonate per liter be used in washing the starch from the flour for the first eleven minutes and that the gluten then be washed in distilled water for two minutes. He found that the same effect could not be obtained with calcium sulfate, calcium chloride, or sodium chloride, all three of these salts giving a lower yield of gluten. Olson (1912a) investigated the yield of gluten obtained when the original flour was doughed up with solutions of acids, bases,

and salts. He found that the general effect was to decrease the yield. The solutions varied considerably in this respect. Upson and Calvin (1916) investigated the effect of washing out the gluten with carbon dioxide–free distilled water, tap water, various concentrations of sodium chloride, dibasic potassium phosphate, hydrochloric acid, and magnesium chloride. They came to the conclusion that carbon dioxide–free distilled water gave the best results. The influence of drying the crude gluten under various conditions was investigated by Neuman and Salecker (1908).

Macfarlane (1905) showed that crude gluten contained considerable other material in addition to protein. The amount of protein dissolved in washing out the gluten with water was determined. The gluten was then washed with 70 per cent alcohol, and the material dissolved by alcohol was determined by loss in weight and by nitrogen determinations of the alcohol extract, and finally the protein in the residue was determined.

Norton (1906), in his investigations of the protein content of crude gluten, found it to consist of only about 75 per cent gluten proteins, the range of protein content in the crude glutens which he investigated being from 68 to about 83 per cent. His results have been confirmed by many other workers. Norton also presented data which showed that the actual weight of the crude gluten varied from about 10 per cent less to over 17 per cent more than the total protein as shown by nitrogen determination on the flour. Olson (1912b) also investigated the composition of crude gluten. Kepner (1914) reviewed the various errors in washing out crude gluten.

We thus see that altho the influence of several factors on the crude gluten determination has been investigated, in many instances the workers arrived at diametrically opposite conclusions. Part of the difficulty may have been that each worker used a different type and grade of flour for his investigations. However, the results point out inherent sources of error in the quantitative determination itself.

While few investigators claim a direct parallelism between the quantity of crude gluten and the baking strength, Jago and Jago (1911) recommended the crude gluten determination and characterize it as follows:

"A gluten determination is an estimation of the amount of those bodies which are in such a physical condition as to impart elasticity and gas-retaining power to the dough at the time when the determination is made."

The principal suggestion of the above investigators regarding the crude gluten determination is that it should be made at the time the fermented loaf is ready for the oven. Balland (1896), while pointing

out that gluten content of flours does not always parallel nitrogen content, nevertheless seems to believe that the gluten content tells the quality of the flour. Fleurent (1901a) points out that hard wheats are richer in gluten than soft wheats. König and Rintelen (1904) find no direct relationship between gluten content and strength. Norton (1906) and Neumann and Mohs (1910) come to the same conclusion.

Unable to find a direct relationship between gluten content and baking strength, Bremer (1907) and Schneidewind (1909) state that the baking strength of flour is dependent upon both quantity and quality of the gluten. Their opinion is shared by many other investigators, the difficulty being in determining the quality. It is the practice in most of the laboratories which make the crude gluten determination to report on both the quantity and the quality of the gluten obtained. The attempt at reporting its quality is usually confined to such descriptive words as tenacious, elastic, inelastic, soft, sticky, etc., the grading depending solely on the worker's judgment and memory.

There have been many attempts to vary the gluten content of a given flour and to compare the loaf volume produced. Snyder (1901) reports the addition of 10 and 20 per cent wheat starch to a given flour without decreasing the loaf volume, altho of course the proportion of gluten in the mixture was decreased. In another set of experiments Snyder washed enough starch from a flour to increase the gluten in one case 11 per cent and in another 25 per cent without materially changing the size of the loaf. Stein (1904) found that adding dry gluten to the extent of 7 per cent increased the volume of the loaf in one instance from 140 to 160 cubic centimeters and in another instance from 144 to 170 and 172 cubic centimeters. Stockham (1920) reports an increase in loaf volume due to addition of gluten. He does not state just how the increase in gluten content was brought about.

In this connection it is interesting to note that Olson (1911) at one time seemed to doubt the necessity of gluten in flour in order to make good bread.

Total protein.—While numerous investigators have shown that the comparison of individual flours for strength on a basis of their protein content is very unreliable, Shutt (1907) (1908) came to the conclusion that there is in general a relationship between baking strength and protein content. Roberts (1920), investigating the protein content of the variety types, finds some relation between type of wheat and protein content. Thomas (1917), in the comparison of total protein content with baking tests, showed clearly, taking the average of hundreds of determinations, an unmistakable relationship between loaf volume and protein content. It seems safe to conclude that taken as a whole there is a relationship between protein content and baking strength,

but such a method of comparison is of little value when it is a question of comparing individual flours.

Gliadin-glutenin ratio.—To Einhof (1805) is attributed the discovery that wheat flour contained an alcohol-soluble protein which he considered the same as gluten. Taddei (1820) found that gluten consisted of two substances, one of which was soluble in alcohol. Since that time many investigations were carried out on the proteins of wheat gluten, namely those by Berzelius (1827), DeSaussure (1833), Boussingault (1837), Leibig (1841), Scherer (1841), Dumas and Cahours (1843), Mulder (1844), Günsberg (1862), Martin (1886), Fleurent (1893), O'Brien (1895), and Ritthausen (1896) (1899), but the most thoro and exhaustive work was that done by Osborne and Voorhees (1893), also Osborne (1907), Osborne and Harris (1905) (1906), and Osborne and Clapp (1906), who showed conclusively that true wheat gluten was composed of only two proteins, i.e., gliadin, soluble in medium concentrations of alcohol, and glutenin, insoluble in alcohol but soluble in dilute acids and alkalies.

No sooner had the identity of the proteins in the wheat gluten been established than investigations were carried out to ascertain the ratio of the two proteins in the glutens of strong and weak flours. Fleurent (1896a) (1896b) (1898a) (1898b) (1905) was one of the early workers to come to a conclusion on this point. He claimed that there was an optimum ratio of gliadin to glutenin of three to one, indicating a strong flour. If the ratio was greater or less than this optimum the flour was weak. He arrived at this conclusion by determining the ratio for different flours and by adding the protein necessary to correct this ratio and finding an improvement. Snyder (1897) (1899a) (1899b) (1901) came to the same conclusion with the exception that he thought the optimum ratio was 65 per cent of gliadin and 35 per cent of glutenin for greatest baking strength. He arrived at his conclusion in much the same way as did Fleurent. Snyder (1901) found that he could increase the gluten content from 11 to 25 per cent, the ratio of gliadin to glutenin remaining unaltered, without making an appreciable change in the volume of the loaf. This led him to the conclusion that it was not the quantity of gluten but the ratio of gliadin to glutenin that was the determining factor. Kosutány (1903) (1910) considers the gliadin-glutenin ratio of importance in determining the baking strength of flours.

Guess (1900) came to the conclusion that the quantity of the gluten as well as its quality as expressed by the gliadin-glutenin ratio was a factor in determining the strength of flour. He proposed that the gliadin-glutenin ratio be called the quality factor and that the actual

quantity also be taken into consideration as a quantity factor. The product of the quality factor times the quantity of gluten represented the baking strength. Norton (1906) comes to nearly the same conclusion. König and Rintelen (1904), Wood (1907a) (1907b), Shutt (1909), Rammstedt (1909), and others came to the conclusion that the gliadin-glutenin ratio was not the determining factor in the strength of flour.

The gliadin-glutenin ratio has fallen into disrepute for another reason, namely, the difficulty of actually determining the true proportions of gliadin and glutenin as they exist in the flour. As early as 1862, Günsberg had shown that gliadin was appreciably soluble in water, and Teller (1896) found that gliadin was appreciably soluble in dilute salt solutions (as concentrated as 10 per cent). After making the corrections for the solubility of the gliadin in salt solution he concluded that gliadin and glutenin occur in the flour in approximately equal amounts. Chamberlain (1904) found that a large part of the water- and salt-soluble proteins were also dissolved in 70 per cent alcohol and would appear as gliadin. Olson (1913) confirmed Chamberlain's results and proposed distilling off the alcohol from the gliadin solution and concentrating to a small volume. This procedure coagulated out the gliadin, which was then filtered off and the amounts were determined, the coagulum being considered pure gliadin.

Bailey and Blish (1915) determined the amount of gliadin extracted from flours by means of water and salt solutions by taking advantage of the fact that the globulin and albumin of flour yield much less nitrogen in the ammonia fraction, when subjected to acid hydrolysis, than does gliadin. By a determination of the ammonia resulting from the acid hydrolysis of the proteins dissolved, they were able to determine the relative proportions of gliadin removed.

Gliadin content.—In addition to the use of the nitrogen determination for the estimation of gliadin, Fleurent (1901b) (1903) found that the gliadin content of a flour could be determined with sufficient accuracy by a determination of the density of an alcoholic solution; while Mathewson (1906a) thought that "the change in density in gliadin solutions for such differences as would be met with in flour analysis would allow rather a narrow limit for experimental error." Kjeldahl (1896), Osborne and Harris (1903), Snyder (1904), Marion (1906), Mathewson (1906b) (1908), Shaw (1907), Lindet and Ammann (1907), Thatcher (1907), Greaves (1911), and others investigated the use of the optical rotatory power of gliadin dissolved in alcohol as a means of its determination. Robertson and Greaves (1911) investigated the refractive index of gliadin in various solvents as a

means of its determination. These methods of determination of gliadin in alcoholic solution have not attained very wide use.

While Greaves (1911) seems to conclude that pure gliadin reaches its maximum solubility in about 70 per cent alcohol, the percentage of protein extracted directly from flour by various concentrations of alcohol seems to go through a maximum at a concentration of alcohol of from 50 to 60 per cent as shown by Snyder (1906), Hoagland (1911), Schleimer (1911), Greaves (1911), Olson (1913), and others.

Chamberlain (1906), in attempting the separate analysis of the salt-soluble and alcohol-soluble proteins from the same sample of flour, confirmed his earlier work and that of Teller (1896)—that alcohol removed a large portion of the salt-soluble proteins. Chamberlain found in addition that if he extracted first with salt solution and subsequently with alcohol, he removed less protein than if he extracted in the reverse order. For example, in a given flour alcohol extracted 7.47 per cent protein, the subsequent extraction with salt solution removed 0.57 per cent in addition, or a total of 8.04 per cent removed by the two treatments. The direct extraction of the flour with salt solution removed 2.18 per cent and the subsequent extraction with alcohol removed 5.50 per cent, or a total of 7.68 per cent. His results point out still more strongly the difficulties encountered in a correct gliadin determination.

Most of the attempts to correlate gliadin content with strength have been based on the amount of protein extracted directly from the flour with 70 per cent alcohol. While König and Rintelen (1904), Norton (1906), Thatcher (1907), Wood (1907a), Rammstedt (1909), and Olson (1917) find no direct relationship between baking strength and gliadin content, nevertheless Norton believed that the best method at hand was the determination of the gliadin content together with the total protein content and the characteristics of the crude gluten, while Shutt (1907) (1909) thought that, in general, there was a relation between gliadin content and strength.

Fenyvessy (1911), attacking the problem in a somewhat different way, found that the loaf volume was increased by the addition of gliadin to flour. He found that rye gliadin would produce the same effect and that most of the added gliadin could be recovered in the crude hand-washed gluten.

Martin (1920) has recently carried out an investigation of some of the properties of wheat flour which he thought might be correlated with strength. He finds for the flours investigated that, "Flours with high gas-retaining capacity and high bakers' marks have been shown to be those in which the 'amended gliadin' figure is high, . . . for flours having a satisfactory gas-producing capacity, bakers' marks,

gas-retaining capacity, and 'amended gliadin' content are closely related, and it is considered that the estimation of either of the latter together with the determination of the gas-producing capacity will indicate the strength of the flour." The amended gliadin figure as used by Martin was determined by subtracting from the percentage of protein extracted from a sample of flour in a Soxhlet extractor by means of 50 per cent alcohol, the percentage of protein extracted from 25 grams of flour by 250 cubic centimeters of water at 25° C., the time of extraction being three hours.

Glutenin content.—Guthrie (1896) has attributed the strength of flour to its glutenin content. He presents data showing that dried glutenin takes up about 78 per cent of its weight of water while dried gliadin takes up only about 40 per cent of its weight. It must be remembered that Guthrie considers the water absorbing capacity of a flour a measure of strength when he says:

"The strength or water absorbing capacity of a flour depends directly upon the relative proportion in which the two proteins are present in the gluten.

"If the gluten contents of two flours are nearly the same, that will be the stronger flour which contains the larger proportion of glutenin.

"Flours in which glutenin preponderates yield strong, tough, elastic, non-adhesive glutens.

"Increased gliadin content produces a weak, sticky, and inelastic gluten."

Woodman (1922), in a recent paper, concludes that the difference between a strong and a weak flour is due to a difference in the glutenin fraction. He bases his evidence on the optical behavior of the pure flour proteins when racemized at 37° with dilute alkali. He finds the gliadin from both types of flour to be identical, but the glutenins possess different optical rotations and when treated with alkali show different racemization curves. Woodman states, "The factor which determines the size of the loaf is most probably connected with the diastatic capacity of the flour as was suggested by this investigator (Wood, 1907a). On the other hand, the factor which determines the shape of the loaf and which appears to be directly related to the physical properties of the gluten of the flour, is possibly dependent on the particular glutenine mechanism possessed by the wheat. The results of this investigation suggest that the strong wheat synthesizes one type of glutenine and the weak wheat a different type." We shall have occasion to refer later to these views of Woodman in connection with our own experimental results.

Fenyvessy (1911) pointed out that the addition of glutenin to flour caused a deterioration in the baking qualities of the flour, altho most

of the added protein could be recovered in the crude gluten determination. He was unable to obtain crude gluten from rye flour to which glutenin had been added. It perhaps might be mentioned here that Nasmuth (1903) stated that glutenin had a definite coagulation temperature of 70° C.

Water-soluble proteins.—While Bremer (1907), Shutt (1909), Rammstedt (1909), and Olson (1917) found no correlation between strength and water-soluble proteins, Rousseaux and Sirot (1913) (1918) believe that the strength of flour bears a direct relationship to the ratio of water-soluble protein to total protein. The ratio for good flours was found to be 1 to 5.72. The flours were considered weak if the ratio fell to 1 to 5.2. The ratio was found to be higher for the higher grades of flour. The ratio varied with the mill streams investigated. They report a ratio of 1 to 8 for a strong, glutenous American flour, and a ratio below 1 to 2 for a sample of flour that had been in storage for a long time.

As a point of interest in this connection, Humphries (1910) made the observation that the addition of a bran extract to the flour in the doughing process improved the quality of the loaf. This effect was later confirmed by White (1913), and Willard and Swanson (1913). Stockham (1920) showed that the water extract from the different grades of flour when used in making the dough for baking, causes an increase in the volume. He states:

"It may be seen that the extract from that grade having the greatest quantity of extract though the weakest when baked alone has the most accelerating and beneficial influence."

Chemical constitution of the proteins from strong and weak flours.—Wood (1907a) found that the amide nitrogen distribution was the same in the proteins of strong and weak flours. Gröh and Friedl (1914) found gliadin prepared from strong and weak flours to be identical in physical properties.

Blish (1916) subjected the individual proteins prepared from strong and weak flours to analysis by the Van Slyke method of amino acid fractionation, and came to the conclusion that as far as the Van Slyke method would show, the proteins from strong and weak flours were identical.

Sugar content.—While it is a well known fact that the sugar content of the flour will affect the resulting loaf in many cases (Wood, 1907a), and that the addition of sugar to flours which ordinarily give loaves of small volume will in many cases cause a great improvement, this phase of the problem will not be considered here, as Shutt

(1908) and others have shown that the sugar content is not the limiting factor and as the sugar content is usually corrected in the experimental baking, especially by the addition of sugar to the dough.

Fat content.—The effect of fat or shortening on the baking properties of wheat flour is a matter of common knowledge. Salmon (1908) found that if he extracted wheat flour with ether and then allowed the flour to dry spontaneously, the loaf made from it was much larger than was one made from the original flour. He also noted that the viscosity of the fatty matter varied within wide limits with the age and variety of the flour. Mohs (1915a) added increasing amounts of fats up to 10 per cent and found the volume of the loaf was increased, different fats varying in their ability to increase volume. Stockham (1920) found that extracting flour with ether caused a decrease in the volume of the loaf. He investigated the effect of adding 0.6 per cent of various fats and fatty acids and found that at this concentration wheat fat was the only one which caused a marked increase in the volume of the loaf while fatty acids all caused a decrease. He noted that the fat from a high-grade flour was relatively more solid than the fat from a low-grade flour. Saunders (1921) reports that the addition of about 3 per cent of lard increased the volume of the loaf on an average of 7 per cent. The effect of other fats and oils was studied.

Phosphorus content.—The relation of phosphorus content to strength has been investigated by many workers. White and Beard (1913), in their study of the problem, determined total phosphorus, organic phosphorus, inorganic phosphorus, and water-soluble phosphorus. They show that in general large loaves have less of all these groups of phosphorus. They concluded that the large loaves contained higher percentages of organic phosphorus than the smaller ones, the values for the larger being 93.5 per cent and for the smaller 87.3 per cent, altho the largest loaf contained only 85.8 per cent and the smallest 94.6 per cent.

Enzymes of wheat flour.—The diastatic enzymes of wheat flour have received much attention. Baker and Hulton (1908), Ford and Guthrie (1908), Swanson and Calvin (1913), and others have investigated their relation to strength. The results of investigations show that weakness in many instances is apparently due to a lack of sufficient diastatic power in the flour to supply the sugar necessary for proper fermentation. To correct this difficulty it is a common baking practice to add malt flours and extracts, and sugars. Koch (1914), as a result of his investigation, concluded that there was no difference in the amount of either diastase or invertase in strong and weak flours.

The proteolytic enzymes, while perhaps playing an important part in the strength of flour, are very difficult to study. Vines (1903) found considerable proteolytic activity in extracts of wheat germ. Proteolytic enzymes are present only in small amounts in flours, as shown by Baker and Hulton (1908), Ford and Guthrie (1908), Weaver and Wood (1920), Stockham (1920), and others. Stockham reports some very interesting experiments which indicate that the proteolytic enzymes found in flour come mainly from the germ. His results also show an increase in proteolytic enzyme activity as the grade of the flour decreases, and in flours made from sprouted wheat.

Gas-producing capacity.—Wood (1906-8) found for a series of flours with which he worked that the gas-producing capacity was closely related to bakers' marks, and believed that the determination of the gas-producing capacity gave an accurate indication of strength. The determination as first recommended by Wood was carried out by measuring over brine the amount of carbon dioxide evolved in 24 hours when 20 grams of flour was treated with yeast and water and incubated at 35° C. While this method has been used by Wood (1907a), Baker and Hulton (1908), Martin (1920), and many other workers, Alway and Hartzell (1909) concluded that the method was of little value. These authors found, in a series of tests extending over a month, in which the gas-producing capacity of the same flour was measured in duplicate 22 times, a difference amounting to as much as 170 per cent in the amount of carbon dioxide evolved in the first hour of fermentation and differences as great as 18 per cent at the end of 22 hours. These authors state:

"There is clearly no direct connection shown between the size of the loaf and the volume of gas evolved. The 13 flours which gave the largest loaves evolved on the average somewhat less gas than the other 13 flours."

Jago and Jago (1911), in their studies of the amount of gas evolved, used whole loaves and found that the strong flours evolved less gas than the weak ones. Stockham (1920) thinks that the amount of gas evolved is related to the grade of flour, the lower grades of flour as a rule evolving more carbon dioxide. The important relationship existing here has perhaps been expressed more clearly by Bailey (1916) when he says, "The strength of flour is determined by the ratio between the rate of production of CO_2 in and the rate of loss of CO_2 from, the fermenting mass of dough." He devised an automatic maximum reading expansimeter for measuring the maximum expansion attained by a given weight of flour in the form of a fermenting dough when treated exactly like another portion which was baked. His results show a close relation between the maximum expansion reached by the dough

and the volume of the loaf produced. Bailey and Weigley (1922), working with a strong and a weak flour, investigated the rate of loss of carbon dioxide from doughs freshly made and from doughs which were normally fermented and ready to go into the oven. Their results show that the rate of loss of carbon dioxide is much greater from the weak-flour dough than from the strong-flour dough, the differences being especially marked in the doughs with no previous fermentation. The rate of expansion is correspondingly greater in the strong-flour dough as compared with the weak-flour dough. Fermented doughs expanded at a much more rapid rate than fresh doughs. To attain a given expansion of dough there was a much greater rate of loss of carbon dioxide from the weak-flour doughs than from the strong-flour doughs.

Hydrogen-ion concentration.—Jessen-Hansen (1911) was the first to point out the influence of the hydrogen-ion concentration on the volume of the loaf of bread produced from a given flour. He found the optimum concentration of hydrogen-ions for baking was approximately at a pH of 5 and that the volume of the loaf may be increased by the addition of acid if the pH without acid was greater than 5.0. In investigating the effect of flour improvers, he came to the conclusion that their effect was mainly to correct the hydrogen-ion concentration of the dough. As a result of the investigations of Jessen-Hansen, and of Cohn and Henderson (1918), Cohn, Wolbach, and Henderson (1918); Cohen, Cathcart, and Henderson (1918), and Henderson (1918) have devised a rapid method for the determination of the acidity of the baked loaf which consists in observing the color produced when a few drops of an alcoholic solution of methyl red are placed on the newly cut slice of bread. Morison and Collatz (1921) cast some doubt on the beneficial effect of acidity on loaf volume.

Mechanical strength testers.—Various mechanical testers have been devised for determining the baking value of flour. One of the first was the so-called "aleurometer" devised by Boland (1849) which consisted in measuring the volume that hand-washed gluten would attain when placed in a cylinder and heated in an oil bath. Kunitz (1885) recommends heating for 20 minutes at 200° C. Foster's gluten tester is an outgrowth of the aleurometer and measures the distance a plunger supporting a definite weight is moved by expanding gluten when baked. Sellnick (1899) devised a small apparatus which operated as an oven and was heated by a given quantity of alcohol in a prescribed manner. Doughs were made, using 20 grams of flour, 0.5 gram of sodium carbonate, 1 gram of acid potassium tartarate, and 18, 20, or 22 cc. of water, and baked immediately, the result giving the relative baking values of the flour. Liebermann (1901) baked the

gluten washed from 20 grams of flour in a hollow capsule which he immersed in an oil bath heated to a temperature of 170° C., and from the volume of the baked gluten he determined the baking qualities of the flour.

Boland, Meyer, Kunis, and Kreusler also developed apparatus designed to determine the strength of gluten of small amounts of flour. The methods devised by these men and others are described by Maurizio (1902), who applied them to several flours. In this connection it is interesting to note that Mohs (1914) devised a mechanical gluten washer.

Wood (1912) used a turbidity method for determining strength. The flour was shaken with water for one hour and a solution of iodine in potassium iodide was added to an aliquot of the filtered extract and allowed to stand for one hour. The depth of the liquid through which an electric bulb could be seen was thought to be related to the strength of the flour. The greater the depth of liquid through which the bulb could be distinguished, the weaker the flour.

Hankóczy (1920) devised an apparatus by means of which a bubble of dough could be blown from a thin dough membrane. He thought baking strength was related to the volume of the bubble and to the pressure the bubble would maintain. Chopin (1921) described a similar apparatus by means of which he measured the tenacity of a dough by determining the maximum size of a bubble which could be blown from a given dough membrane. He found a direct relation between the square root of the volume of the bubble and the volume of loaf which a given flour would produce.

Cytology.—Miss Brenchley (1909) investigated the structure of the wheat kernel and its relation to strength. She concludes from her investigation that cytologically the wheat kernels that yield strong flours do not differ from those that yield weak flours.

Colloid chemical investigations of strength of wheat flours.—With the comparatively recent development of colloid chemistry, particularly the study of emulsoids, the attention of investigators on the subject of flour strength has been turned to the study of the colloidal factors that might influence the baking quality of flour.

Balland (1883) (1884), altho apparently not realizing the significance of his work from the colloid viewpoint, showed that gluten triturated with saturated sodium chloride lost some of its weight and attained the consistency of rubber. By washing in distilled water the gluten again took up the water which it had lost and attained its original weight, altho all the salt was not removed, as the ash was 2.8 grams, the ash of an untreated portion being 0.154 grams. He

found solutions of the following salts to act similarly to sodium chloride, but with greater or less intensity: ammonium acetate, potassium carbonate, copper sulfate, iron sulfate, magnesium sulfate, zinc sulfate, alum, and glycerine. After immersion in 1-10 solutions of potassium bromide, potassium iodide, potassium nitrate; and 1-20 solutions of potassium chlorate and potassium borate; and in olive oil for 24 hours, the gluten did not appear to be modified. The gluten which was placed in the following solutions was attacked more or less and disappeared completely by washing in an excess of water: dilute nitric and hydrochloric acids, 1-10 citric and tartaric acids, dilute alcohol, dilute ammonia water, crystalline lead acetate, lead subacetate, and mercuric nitrate. He dried 100 grams of gluten in air and found that it lost 56 grams of water. In washing in water it took up all the water it lost and attained its original elastic properties. A similar sample dried at 100° C. lost 65 grams of water, but when washed in water it took up only a part of the water lost and its elastic properties had disappeared. Stein (1904) tried the effect on the yield of crude gluten of mixing various salts with the flour.

Wood (1907a) (1907b), Wood and Hardy (1908), and Hardy (1910) were the first to apply the methods used in the study of emulsoid colloids to the gluten from wheat flour. Hofmeister (1890) and many others had previously studied the effect of acids, bases, and salts on the imbibition of typical emulsoids.

Wood (1907b) extended these studies to the gluten from wheat flour. He was the first to attribute the differences between strong and weak flours to the colloidal properties of the proteins rather than to the chemical properties. This conclusion was reached only after a careful investigation of the chemical properties, as noted previously, such as: total nitrogen, total gliadin nitrogen, amide nitrogen in the gliadin, ratio of gliadin to total protein, total ash, acid, and carbon dioxide produced in the different flours.

In their study of the effect of acids, bases, and salts on the gluten, Wood (1907b) and Wood and Hardy (1908) suspended strings of gluten across V-shaped glass rods and immersed these strings of gluten in beakers containing the solutions that were being investigated. They found that if the gluten was suspended in distilled water it retained its coherence almost indefinitely, but if suspended in 0.001 normal hydrochloric acid, it immediately began to disperse, the rate of dispersion increasing as the concentration of the acid increased up to about 0.033 normal. As the concentration of acid increased from this point the dispersion rate decreased until the gluten finally became more coherent in 0.083 normal hydrochloric acid than it was originally. It was noticed that with sulfuric, phosphoric, and oxalic acids, dispersion

took place even in high concentrations. Wood (1907b) also noticed that if salts were added to the acid solutions the effect of the acid was in a large measure counteracted. He concludes from his experiments that "the variations in coherence, elasticity, and water content observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of the glutens themselves."

Olson (1912a) studied the effect of modifying the gluten environment of the flour on the crude gluten determination. The method he used was to dough up 10 grams of the flour with 6 cubic centimeters of the solution being studied. After standing for one hour the gluten was washed out with distilled water and the results were tabulated under the heads of percentage of gluten, weight of nitrogen in the gluten, percentage of nitrogen in the gluten, percentage of total nitrogen, and percentage of total nitrogen calculated from the nitrogen in the gluten from the original flour as 100.

These determinations were carried out with the original flour, with flour that had been made up into a dough and then dried and remilled, with flour that had been made up into a dough and then dialyzed for about three days with frequent changes of water and then dried and remilled, and finally with flour that had been stirred with a large volume of water and allowed to settle and the supernatant liquid decanted. After this process was repeated several times, the material was dried and remilled. He found that by mixing the untreated flour with 0.10 normal solutions of acids, salts, and alkalies the yield of gluten decreases, the effect being in the following order, sodium phosphate having the least effect: sodium phosphate, sodium chloride, magnesium sulfate, potassium phosphate, calcium phosphate, aluminum sulfate, sodium hydroxide, potassium hydroxide, sulfuric acid, phosphoric acid, and hydrochloric acid. It was found that the mere doughing up and drying process changed the character of the gluten to a small extent. The glutens that had been dialyzed showed marked differences from the original flour, the gluten yield being smaller. In the case of the decanted flour, it was almost impossible to collect the gluten, and values were obtained only in the case of sodium and potassium hydroxide, sodium sulfate, and magnesium sulfate. The gluten could not even be collected when treated with water only. Olson states that acids have a greater prejudicial effect than alkalies, and that of the alkalies, calcium hydroxide has a greater prejudicial effect than sodium or potassium. He concludes "there is as yet some unknown substance which is important in causing a transformation of the physical properties of the gluten."

Gröh and Friedl (1914) made a careful study of the physical properties of gliadin separated from strong and weak flours. They studied the following physico-chemical properties of gliadin solutions of the different flours in potassium hydroxide: viscosity, surface tension, specific rotation, gold number, refractive index, and the time it took a platinum sol to which a given amount of gliadin had been added, to decompose half of a known amount of hydrogen peroxide. They separated the alcohol-soluble protein of flour into four fractions and found them all to be the same within experimental error except the first, which was shown to contain impurities. They show that the above physical constants of the gliadins separated from four flours of different strengths are the same. They conclude that the differences in strength are colloid-chemical and that these differences are changed either by the extraction with alcohol or by the treatment with potassium hydroxide. They obtained from rye an alcohol-soluble fraction which appeared to be identical with wheat gliadin in the above mentioned physico-chemical properties.

Upson and Calvin (1915) (1916) studied the imbibition of gluten as influenced by acids, and acids in the presence of salts. A more exact method was used than that of Wood and Hardy. The gluten was washed from flour and pressed into sheets from which discs were cut with a large cork borer. The discs were then weighed and placed in the various solutions for a given period of time, when they were removed and again weighed. The results were calculated on the basis of increase in grams per gram of moist gluten. They confirmed the findings of Wood and Hardy, finding the greatest imbibition in the case of hydrochloric acid at moderate concentrations (0.01 normal), while with high concentrations (0.2 to 0.5 normal), the solutions actually removed water from the gluten. They found that with lactic and acetic acids this effect was not so marked at the higher concentrations, in the case of the lactic acid the swelling being almost as great in 0.5 normal as in 0.005 normal solutions. They also found that in the presence of acid plus various salts the swelling decreased almost in proportion to the amount of added salt. They also demonstrated the reversibility of the action. Discs that had more than doubled in weight after remaining in acetic acid for two hours, not only resumed their original weight but also their original appearance and physical properties of toughness and elasticity, when placed in 0.1 normal dipotassium phosphate for one hour. Upson and Calvin (1916) conclude "that the bread making qualities of dough made from wheat flour are dependent on the quantity and quality of the contained gluten. Quality of gluten is regulated by the kind and concentration of the acids and salts present in the dough. If the kind and amount of

acids and salts are such as to favor water absorption, the quality of the gluten will be poor, whereas the presence of acids and salts in such amounts as to tend to inhibit water absorption makes for an improved gluten."

Gortner and Doherty (1918) applied the method used by Upson and Calvin to gluten separated from flours of different strengths. The other workers in this field had worked with only one type of gluten and had drawn conclusions for the other types. Gortner and Doherty found that glutens from strong flours had a greater rate of imbibition than glutens from weak flours. This difference was noted in solutions of hydrochloric, acetic, lactic, orthophosphoric, and oxalic acids. They tried the effect on the swelling rate of five different glutens in various concentrations of these acids alone; and in the case of three of the glutens, the effect of hydrochloric and lactic acids in the presence of 0.005 molar solutions of potassium chloride, potassium phosphate, potassium tartrate, calcium chloride, mercuric chloride, aluminum sulfate, and magnesium sulfate; and in addition the effect of the last named three salts on swelling with oxalic and acetic acids. While the effect of the addition of salts to the various acids was to decrease the imbibitional rate, the ratio of the effect on the strong and weak glutens remained about the same, that is, the strong flour glutens still had a higher rate of imbibition than the weak flour glutens. This is the first instance in which a marked difference in physico-chemical properties has been found between the glutens from strong and weak flours, except, of course, in the general appearance of the gluten. Gortner and Doherty show that their results refute the conclusions of Upson and Calvin regarding flour strength in that the crude glutens investigated by both Gortner and Doherty and by Upson and Calvin contained almost the same amount of moisture. This would not be the case if the conclusion of Upson and Calvin were true. The differences in imbibitional power shown by the glutens that Gortner and Doherty used are so great that they could not possibly be correlated with the original moisture content of the wet gluten. These authors show that in addition to a marked difference in the rate of imbibition, there is a marked difference in the maximum hydration capacity, the weak-flour gluten dispersing much sooner and at a much lower water content than the strong-flour gluten. These results also refute the contention of Wood and Hardy (1908) that the difference between them is due to the strength of the electric double layer. If this were true the glutens would all have the same imbibitional rate but it would be found in different concentrations of acid.

Gortner and Doherty conclude that the glutens are different even at the isoelectric point and that, "the difference between a strong and

weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which the properties are much less marked. It is suggested that such differences may be due to the size of the gluten particles and that at least a part of the particles comprising the weak gluten may lie nearer the boundary between the colloidal and crystalloidal states of matter than is the case with the stronger glutens."

Henderson, Fenn, and Cohn (1919) report some very interesting studies of the effect of electrolytes on the viscosity of dough. The method used was to prepare a dough from a given flour, using a constant volume of water or solution. Twelve grams of this dough were placed in a small chamber with pins protruding from the bottom to hold the dough firmly, a paddle wheel was thrust into the dough and rotated by means of a weight on the end of a string which passed over a pulley, and the time taken for this weight to move through a distance of 2 meters was used to indicate the viscosity of the dough. In studying the effect of hydrogen-ion concentration, it was found that the viscosity of the dough passed through a minimum occurring at approximately a pH of 4.7. By adding increasing concentrations of sodium chloride to the dough the viscosity first fell and then rose to above its original value. Magnesium sulfate caused a greater fall over a longer range of concentration and a slight rise at the higher concentrations. The effect of various concentrations of sodium chloride at a pH of 5.8 and of 4.8 was investigated. The viscosity fell more at 4.8 and the rise in the higher concentrations of salt was very slight. By using a constant quantity of the various salts and varying the hydrogen-ion concentration, the minimum viscosity values were found at widely different hydrogen-ion concentrations. The minimum with sodium chloride was found at a pH of about 3.8, while with calcium chloride the minimum was at 5.8. The authors state, "It is advantageous to employ a dough of relatively low viscosity or, speaking more correctly, to reduce whatever quantity of gluten may be present to a condition in which the viscosity is low."

In a later paper Henderson, Cohn, Cathcart, Wachman, and Fenn (1919) report some of the results of their study on the conductivity, hydrogen-ion concentrations, and binding power of gluten in the presence of acid or alkali. These authors conclude, "The results seem to show that simple chemical phenomena are most important in such systems and that the modification of these resulting from colloidal and heterogeneous characteristics are of secondary importance in determining the condition of equilibrium tho somewhat significant in the progress of the system toward the condition of equilibrium."

Mohs (1915b), in a general theoretical discussion, points out the importance of the different phases, especially the relationship of the surface involved and the resulting power of adsorption. He emphasizes particularly the behavior of gliadin as a protective colloid. He explained that the gluten in the dough is in the gel state, in the flour it was said to be present in a more dehydrated form, while if the flour was heated the gluten was irreversibly coagulated and would not again take up water to form gluten.

Ostwald (1919), Lüers (1919), and Lüers and Ostwald (1919) (1920a) (1920b) investigated the problem of bread making from the colloid standpoint. Ostwald (1919), in the first paper of the series, gave a general discussion of the bread baking process from the colloid viewpoint. He said that flour is a coarse dispersion of several poor-in-water hydrogels, such as protein gel, carbohydrate gel, the starch grains, and cellulose gel of the cell walls (crude fiber). There are also present the molecularly dispersed substances, salts, sugar, acid, and especially water, also gases not only dissolved but adsorbed on the surface. The dough is described as a polydisperse system where water is the dispersion medium in which dextrin, sugar, alcohol, and especially salts, acids, and gases are molecularly dispersed, the dissolved plant albumin, swollen starch grains, and finer gas bubbles are colloidally dispersed, and finally the yeast cells and lactic acid bacteria make up the coarse dispersed part. The dough has a combination of the properties of a solid and a liquid. In the baking process the starch becomes gelatinized and the protein coagulated, the carbon dioxide and some of the water are driven from the molecular dispersion over into a gas dispersion which becomes fixed in the gel sponge structure of the bread. Ostwald suggests that the staling of bread might be due to syneresis. He also points out many lines of investigation in which viscosity determinations under certain conditions would throw light on the problem of bread baking.

Lüers and Ostwald (1919), in the second paper of the series, began a study of the viscosity of the flour paste made by heating the flour with water at 100° C. They considered that there were two great regions offered for viscosity study, the one which has to do with dough formation and the other with dough baking. They believed the properties making for a good dough and those making for good baking conditions are distinct. The method used in preparation of the flour pastes was first to dough up the accurately weighed amount of flour with small amounts of water, finally adding a total of 10 cc. This material was poured into 100 cc. of boiling water and heated one minute, and then filtered through muslin into a tared 500 cc. Erlenmeyer flask, washed, and made up to a weight of 200 grams, closed with a

stopper carrying a capillary tube, placed in a boiling water bath for half an hour, cooled to 20° C., and the viscosity determined in an Ostwald capillary viscosimeter. The viscosimeter used had a capacity of about 10 cc. and the time of outflow for water was about two minutes.

Using this method it was found that boiling from half an hour to two hours decreased the time of outflow from 1098 to 1079 fifths of seconds. When working with the lower concentrations it was found that if a more concentrated solution was first made up and then diluted the readings were much higher than if the more dilute solution was made up directly. The viscosity of the diluted solution eventually fell to a value nearly the same as the one made up directly without previous dilution. The explanation offered for this phenomenon is that in more concentrated flour pastes larger particles are present and the dilution causes an increase in the degree of dispersion. In an investigation of the different grades of flour, these authors found the viscosity of flour pastes divided themselves into three classes; the patent flours showed a greater viscosity than the straight flours, while the first clear flour showed the least viscosity. This was found to hold for both the wheat and the rye flours investigated. They concluded that the viscosity of the flour pastes was directly proportional to the starch content and suggested that the viscosity of the starch pastes might be used as a method of measuring the starch content of flours. It was found that the addition of increasing concentrations of sulfuric acid caused an increase in viscosity amounting to from 7 to 9 per cent, followed by a fall in viscosity. They attribute this effect to the protein present and not to the starch, since Samec (1912) (1914) and Samec and von Hoefft (1913) have shown acids to have the reverse effect on pure starch pastes.

In the measurement of the viscosity of the dough solutions, Lüers and Ostwald used a viscosimeter of the Ostwald type having a capillary 3.5 millimeters in diameter and a capacity of about 20 cc. The time of outflow for water was 24 fifths of seconds. The dough solutions were prepared by adding to the flour one and one-half times its weight of water and stirring to a paste, then adding water from a burette until the desired amount had been added. These authors investigated solutions ranging in concentration from 7 to 20 per cent flour. They found that rye flours of the same grade and concentration gave relatively much more viscous solutions than did wheat flours, and this is suggested as a method of differentiating the two. With wheat flour the lower the grade the more viscous was the solution, just the reverse of the behavior of the flour pastes prepared by heating the flour in water. With rye flours, the same rule holds except that the clear and the 94 per cent flour both showed a low viscosity.

They attribute this to the presence of more of the proteins from the aleurone layer which they think have a depressing effect on the viscosity. It was found that the viscosity of the wheat flour solutions decreased with time except in the 40 per cent patent in a flour-water suspension of 13.05 per cent (the highest investigated) in which there was a slight increase followed by a decrease. With rye flours, however, there was an increase with time of standing in all cases amounting to 13 per cent in the 40 per cent rye patent in a flour-water suspension of 13.05 per cent, the highest investigated. They were unable to find in the literature any instance in which this after-swelling had been taken into consideration.

The investigation which these authors carried out on the effect of various concentrations of lactic acid on the rye and wheat flours of different grades is so interesting that some of their data are grouped in Table I.

TABLE I

EFFECT OF 0.050 NORMAL SOLUTIONS OF LACTIC ACID ON RELATIVE VISCOSITY (WATER TAKEN AS UNITY) OF 20% SOLUTIONS OF WHEAT FLOUR OF DIFFERENT GRADES AND OF 0.015 NORMAL SOLUTION. OF LACTIC ACID ON 6.98% SOLUTIONS OF RYE FLOURS OF DIFFERENT GRADES. TEMPERATURE 20° C.*†

Mill grade per cent extraction	20 per cent wheat flour solutions		6.98 per cent rye flour solutions	
	Viscosity in water	Viscosity in 0.050 N lactic acid	Viscosity in water	Viscosity in 0.015 N lactic acid
0-40	3.54	16.16	2.17	2.00
0-60	4.24	22.29	3.33	3.02
0-75	4.54	11.04	3.96	3.88
0-90	4.71	7.67	4.38	4.08
0-94	5.13	7.70	2.88	2.64
60-90 clear	9.67	9.79	2.29	2.13

* Data taken from work of Lüers and Ostwald (Kolloid Z. Bd. 25, S. 126-127).

† Intermediate concentrations were also investigated but the differences are most apparent in the values selected for this table. In the case of the 75% rye flour, a rise to 4.46 was found in lactic acid of 0.0038 normality.

With wheat flours, except the 40 per cent patent, the effect of acid in increasing the viscosity diminishes as the grade decreases. One of the most striking results of this study is the difference in effect of lactic acid on the rye and the wheat flours. Acid seems to have relatively no effect in increasing the viscosity of rye flour solutions.

These authors investigated the hydrogen-ion concentration of the solution filtered from 7.5 per cent dough solutions of various grades of flour when made up with water and when made up with 0.0038 normal lactic acid, and found that the higher the grade the more acid was the water solution, also the higher the grade the more acid was the lactic acid solution. The actual hydrogen-ion concentration of the water extract of rye and wheat flours of the same grade was nearly

the same. The spread of the difference of hydrogen-ion concentration between water and lactic acid solutions decreased with the grade. A more thoro study of this point has been carried out by Bailey and Peterson (1921) who found a close relationship between the buffer value of the water extract and the grade of the flour, the lower grades having the greatest buffer value.

Lüers and Ostwald studied the effect of sodium chloride on the viscosity of the different grades of flour and found that it decreased the viscosity. In their study of the effect of the various hard waters they found that some of them raised the viscosity while others lowered it. In one instance a weak rye flour of 94 per cent extraction was compared in viscosity with the 94 per cent rye flour previously used and was found to have only about two-thirds the viscosity of the latter. It was found that the addition of salt to the concentration used did not decrease the viscosity of the weak flour.

In the third paper of this series, Lüers (1919) took up a study of the gliadin prepared from wheat and rye flours. He studied the effect of acids, bases, and salts on the viscosity of gliadin sols. In each experiment, 15 cc. of carbon dioxide-free distilled water was placed in the viscosimeter and 2 cc. of an alcohol solution of gliadin of a known concentration was added. The outlet tubes of the viscosimeter were protected from carbon dioxide with soda lime tubes, and air was blown through the apparatus to stir the solution. The effect of the electrolyte being studied was carried out by adding small amounts of a concentrated solution to the material already in the viscosimeter, corrections being made for the dilution. The reason for guarding against carbon dioxide was that he found gliadin to be precipitated by the trace of carbon dioxide ordinarily present in the distilled water. Gliadin was, however, found to dissolve in water saturated with carbon dioxide. It was found that as increasing amounts of hydrochloric acid were added the viscosity of the gliadin solution decreased slightly, then increased to a maximum and again fell to nearly its original value. With sodium hydroxide the viscosity rose to a maximum and remained near the maximum point as far as investigated. The maximum for the sodium hydroxide was not nearly so high as for the hydrochloric acid. It was found that hydrochloric, sulfuric, and lactic acids at low concentrations all precipitated gliadin. The increase in viscosity produced by increasing concentrations of sulfuric acid was not great and gliadin was precipitated at the higher concentrations. Lactic acid caused a greater rise in viscosity than did sulfuric acid and produced only a slight turbidity at the higher concentrations. The maximum viscosity for hydrochloric acid occurred at a normality of 0.0004 for wheat, and 0.00066 for rye gliadin. The maximum viscosity obtained

with sulfuric acid occurred at a concentration of 0.0022 normal for both wheat and rye gliadin. With lactic acid the values were 0.018 and 0.011 normal. With sodium hydroxide the maximum was found to be at 0.0022 normal for both, while the maximum for the wheat gliadin in barium hydroxide was at 0.013 normal and for rye gliadin 0.014 normal.

Salt solutions were found to have a depressing effect on the viscosity in the presence of lactic acid. Hydrochloric acid was found to have a marked increasing effect on the viscosity of the gliadin when dissolved in 71.5 and 42.6 per cent alcohol. The viscosity in each case passed through a maximum at 0.0015 normal hydrochloric acid. It was shown that the viscosity increased with rise in temperature. Diluting the gliadin solution with water caused an increase in viscosity, but if 0.05 cc. of 0.5 normal hydrochloric acid had been added previously, the viscosity decreased with dilution. The author explained the rise in the first instance as being due to dilution of the alcohol which permitted a hydration of the gliadin by water, while with hydrochloric acid of the concentration mentioned no hydration could take place. In all the experiments reported, no essential difference was noted between the gliadin from wheat and that from rye and the author concluded that they were identical. This confirms the earlier conclusions of Gröh and Friedl (1914).

Lüers reported the results of three cataphoresis experiments. At a pH of 9.05 the gliadin migrated to the anode, at 6.70 there was no apparent migration, and at 5.98 the migration was to the cathode. The coagulation optimum was found near a pH of 6.15. He concluded that the isoelectric point of gliadin is near the neutral point. Rona and Michaelis (1910) found the isoelectric point of gliadin to be at a hydrogen-ion concentration of 6.0×10^{-10} or a pH of approximately 9.2. Lüers found that gliadin bound more base than acid. The author concluded that the hydrosol of gliadin was a suspensoid because it was so sensitive to electrolytes, while in alcohol gliadin was an emulsoid. He found the precipitate obtained at the isoelectric point was different in character from the precipitate obtained in high concentrations of acid. He considered that gliadin would be in a slightly swollen condition at a pH of 5.0, which Jessen-Hansen (1911) had shown to be the optimum for bread making. Lüers seemed to consider gliadin the most important colloid of the gluten and emphasized its value as a protective colloid, yet he mentioned that glutenin must also play an important part.

In the fourth paper, Lüers and Ostwald (1920a) reported the study of the viscosity of two weak rye flours. They found that one has about one-fourth the viscosity of the normal rye flour of the same

grade. It is emphasized that the best flour should probably show a medium viscosity, neither too high nor too low.

In a fifth paper, Lüers and Ostwald (1920b) discuss the work of Upson and Calvin (1916). They state that their conclusions are in essential agreement. While both emphasize that it is the quality and not the quantity of the gluten which is important, they seem to think that the quality is regulated by the acids and salts present in the flour, or those added to it.

In a later paper, Lüers and Schneider (1921) discuss the methods of measurement of the swelling of colloids as applied to the proteins of flour. They found that by using the viscosity and sedimentation method as affected by acid and alkali they obtained results which when expressed graphically gave curves similar to the ones obtained by Upson and Calvin (1916) for the swelling of gluten discs by the weight method. These curves were also similar to the ones obtained by Lüers (1919) for the viscosity of gliadin. They conclude that all three methods accomplish the desired object.

Whymper (1920) emphasizes the importance of the starch in the colloid chemistry of bread making.

Stockham (1920) determined the concentration of various salts necessary to overcome the dispersing effect on gluten of several acids in different concentrations. He also states ,"Gluten may easily be obtained in a coherent form from the dispersed state but attempts to secure gluten in this manner identical in properties with the original were not satisfactory."

Sharp and Gortner (1922) in a continuation of work along the line begun by Gortner and Doherty (1918) found, in a study of the imbibitional properties of the glutens from four different flours, that the strong flour gluten had a much higher rate of imbibition than the weak flour gluten. Sharp and Gortner found this difference in rate held in solutions of various concentrations of hydrochloric acid, lactic acid, and potassium, sodium, barium, calcium, and ammonium hydroxides. They conclude that the presence of salts decreases the imbibition in alkalies in somewhat the same manner as in acids. They find the reaction of glutens in alkalies to be somewhat different from the reaction of the glutens to acids, dispersion taking place much more rapidly and at lower concentrations in the case of alkalies.

They state, "Drying the glutens washed from the different flours in a vacuum oven at 45-50° C. markedly altered the physico-chemical properties of the glutens, the properties of the different glutens studied becoming more nearly alike. This conclusion is in complete accord

with the theory that the strong gluten is strong because of more pronounced colloidal properties, inasmuch as it is well known that alternately wetting and drying a colloidal gel breaks down the gel structure."

Sharp and Gortner present evidence tending to show that the optimum hydrogen-ion concentration for the imbibition of discs of gluten is the same for the various acids. They conclude that their findings are in complete accord with the earlier postulate of Gortner and Doherty (1918).

Mohs (1922) makes a three-fold division of the water content of the gluten of flour: (1) The lowest, which is found in flour that has been heated; (2) that which is found in normal flour (in this division the "hard" wheat gluten contains less water than the "weak" wheat gluten); (3) that obtained when flour is mixed with water (in this division the weaker the flour the greater the quantity of water absorbed by the gluten). This representation is in close agreement with the explanations of Upson and Calvin (1916) who in addition believe that a special combination of electrolytes causes the gluten to take up more water and thus behave as a weak gluten. Gortner and Doherty (1918) did not agree with the explanations of Upson and Calvin.

Mohs thought that the gluten particles of the strongest flours adsorbed the water on their surfaces but that no water penetrated them. Such flours were too strong. In the case of the normal flour it was explained that part of the water was adsorbed on the surface and part entered the gluten particles, causing them to swell; while with the weak flour gluten the water all entered the particles with none or relatively little on the outer surface, and this hydration of the interior had a great tendency to cause the disintegration of the particles.

This review of the main lines of investigation on flour strength seems to indicate that the greatest advance toward a solution of the problem has been made in the investigation of the colloidal properties of flour.

In taking up a further study of the colloidal properties of the strong and weak flours, the method previously used by Sharp and Gortner (1922) and others, of washing out the glutens and studying their imbibitional rates, was abandoned for the much simpler procedure used by Lüers and Ostwald (1919), which consisted essentially in determining the viscosity of a flour-in-water suspension.

Sharp and Gortner (1919) had found that when glutens were dissolved in various concentrations of potassium hydroxide the gluten from a strong flour exhibited a greater viscosity than that from a weak flour. The concentration of dispersed gluten was ascertained by means of a Kjeldahl determination on an aliquot of the potassium hydroxide solution.

VISCOSITY OF COLLOIDAL SYSTEMS

In the following discussion no attempt is made to review all the literature on the viscosity of colloids. The discussion is intended rather to indicate the special applications of viscosity measurements to colloidal systems, and to show how viscosity measurements have bene made to serve as an index to the changes taking place in such systems.

Coefficient of viscosity.—The coefficient of viscosity in the centimeter, gram, second system may be defined as that force which when exerted on unit area between two parallel planes of unit area placed one centimeter apart would produce a difference in the velocity of streaming of the two planes of one centimeter per second. The reciprocal of the viscosity is called the fluidity.

It was suggested by Deeley and Parr (1913) and later recommended by Bingham and Jackson (1917) that the unit of viscosity be called the "poise" to commemorate Poiseuille's pioneer work in the field of viscosity studies. It is also convenient to use the sub-multiple of this unit which therefore is called the "centipoise." It so happens that one centipoise is almost exactly the absolute viscosity of water at 20° C. The value given by Bingham and Jackson (1917) is 1.0050 centipoise.

Methods of measurement.—The methods used for the measurement of viscosity are modifications of a few general types. The two types used in this investigation will be discussed in some detail. The first of these is the method studied first by Poiseuille (1847), who developed from the results of his measurements the equation for the flow of a liquid through small capillary tubes. The equation as developed by Poiseuille was

$$V = K \frac{p D^4 t}{l}$$

in which V was the volume of the liquid which flowed in the time t, through a capillary whose length was l and whose diameter was D, p being the pressure causing the flow and K being a constant. This equation was developed further by Hagenbach (1860) who expressed the relationship in the following form:

$$\eta = \frac{\pi r^4 p t}{8 l V}$$

Where η is the coefficient of viscosity, r the radius of the capillary, l its length, V the volume passing through the capillary under a pressure p, in the time t. We see from these two equations that for

a given volume of liquid and a given capillary the time of outflow will be inversely proportional to the pressure causing flow. This, in brief, is Poiseuille's law. This equation has been found to hold fairly well for pure liquids within normal ranges of pressure and for small capillaries whose length exceeds a minimum value. In the more accurate work a correction for kinetic energy is introduced. The following equation is given by Bingham and White (1912):

$$\eta = \frac{\pi\ g\ r^4\ p\ t}{8\ V\ (l + \lambda)} - \frac{M\ \rho\ V}{8\ \pi\ t\ (l + \lambda)}$$

Where η is the coefficient of viscosity, g the gravitational constant, p the pressure, t the time taken for the volume of liquid V to flow through the capillary of length l and radius r. ρ is the density of the liquid, M is a constant to which the author assigns the value of 1.12, and λ is a correction to be made to the length of the capillary on account of viscous resistance outside the capillary.

Reynolds (1883) found that, for large tubes, as soon as the pressure forcing the liquid through the tubes exceeded a certain value, Poiseuille's law no longer held and the movement of the liquid through the tube instead of being smooth became turbulent. Glaser (1907) investigated the effect of the diameter and the length of the capillary on the validity of Poiseuille's law and found that for very viscous substances Poiseuille's law held, while with less viscous substances it did not.

Bose (1911), Bose and Bose (1911), Sorkau (1911), and others also investigated the effect of pressure on flow through capillary tubes. Sorkau (1912) explained the flow of liquids through a capillary in the three states of turbulence as follows: Turbulence No. 1 was the ordinary flow in which Poiseuille's law holds. Turbulence No. 2 was a transition condition between Turbulence Nos. 1 and 3. Turbulence No. 3 was a condition which exists at higher pressures. In this case the liquid moves through the capillary more or less like a solid rod, and between the column of liquid and the wall a portion of the liquid forms a smooth sliding surface.

While there are numerous forms of capillary viscosimeters, perhaps the Ostwald type is the best known. In using this apparatus the time of outflow for a liquid whose viscosity is known must first be determined, then the viscosity of any other liquid may be determined from the equation, knowing the time of outflow and the densities.

$$\frac{\eta}{\eta_1} = \frac{D\ t}{D_1\ t_1}$$

Where η and η_1 are the viscosities of the standard and unknown liquids respectively, t and t_1 are the times of outflow and D and D_1 are the densities. For very refined work, Bingham and White (1912) suggest a correction for differences in kinetic energy.

A second type of instrument has been devised for the determination of the viscosity of a liquid by a torsional balance. This method appears to have been first used by Coulomb (1800). A disc, ball, or cylinder is suspended by means of a fine wire in a cylinder containing the liquid being investigated. In instruments of the Doolittle (1893a) (1893b) type the inner ball is usually rotated through one revolution and then released. The damping effect of the liquid on its rotation indicates the viscosity, the more the rotation is retarded the higher the viscosity of the liquid. In instruments of the Couette (1890) type the outer cylinder is rotated at a constant speed until the torsional force exerted on the wire by the suspended cylinder balances the viscous resistance of the liquid, and remains in a fixed position. The angular displacement is then read off on some convenient scale. In instruments of this type care is taken to eliminate the effect of the ends, as for example, the apparatus, used by Perry, Graham, and Heath (1893), Drew (1901), Hatschek (1913a) (1913b), and others, which permits the calculation of the viscosity coefficient from the dimensions of the apparatus.

MacMichael (1915) devised an apparatus of this type which has been used more or less in the United States. In this instrument a disc was suspended in a cylinder containing the liquid being investigated. It was necessary to calibrate the instrument with a solution of known viscosity, in order to express the results in absolute units.

Hays and Lewis (1916) devised an apparatus quite similar to the MacMichael viscosimeter.

Herschel (1920), of the Bureau of Standards, in a study of the MacMichael viscosimeter found it impossible to calculate the viscosity of a liquid from the dimensions of the instrument. In an attempt to more nearly approach ideal conditions, a modification of the MacMichael instrument was developed by Eimer and Amend in which a cylinder of 1 centimeter diameter suspended in an outer cylinder of 3 centimeters diameter was substituted for the disc in the old instrument. The lower end of the inner cylinder was concave so that a bubble of air was caught in the recess, thus eliminating the lower end. The outer cylinder was rotated at a constant speed of 19.1 revolutions per minute. These conditions thus simulate very closely the definition of the unit of viscosity in which the planes are 1 centimeter apart and the difference in velocity is 1 centimeter per second.

Viscosity measurements have attained a wide use in the testing of lubricating oils. Technical viscosimeters have been devised for this purpose by Saybolt, Enger, Redwood, Tagliabue, Dudley, Scott, Doolittle, Magruder, and others. These instruments and the methods of use are all described by Gill (1910) as chairman of a committee of the American Society of Testing Materials on standard tests for lubricants.

Equations for the viscosity of colloidal systems.—Einstein (1906) (1911), Hatschek (1910) (1911), Arrhenius (1916), Smoluchowski (1916), and Hess (1920a) have developed equations for the viscosity of colloidal solutions based more or less on theoretical considerations. These equations all express the viscosity of the colloidal systems in terms of the viscosity of the dispersion medium and some relationship with the volume occupied by the dispersed phase.

Bingham and Durham (1911) found that the decrease in fluidity of clay suspensions was directly proportional to the volume concentration of the dispersed phase. Bingham (1916), in a further study on clay suspensions, showed that as the concentration of the clay increased, the system passed from the viscous to the plastic flow. In order to cause the flow of plastic substances, the shearing force must exceed a definite minimal value. This minimal value he calls the friction constant. In his study of plastic flow through capillary tubes, Bingham found that if the friction constant was subtracted from the pressure producing the flow, within certain limits, the volume of flow was then directly proportional to this difference. He thus showed that there were two factors entering into the flow of plastic materials, namely, the friction constant and the mobility. Bingham (1922) has recently published in book form a discussion of fluidity and plasticity.

Special factors which influence the viscosity of colloids.—The viscosity of pure liquids and molecular solutions was found to be independent of the rate of shear within the lower limits. Even at extremely low rates of shear, Duff (1905), Gurney (1908a), and Griffiths and Griffiths (1921) found that Poiseuille's law held. This was not found to be the case with some colloidal solutions, as shown by Hess (1910) (1912a) (1912b) (1920a) (1920b), Hatschek (1913a), Rothmann (1914) and especially Rothlin (1919), the colloidal solution always becoming less viscous at the higher rates of shear.

Rothlin, using the horizontal capillary tube method, found that for a 1 per cent gelatin sol at 15.5° C. the deviations from Poiseuille's law were greater the longer the solution stood after preparation. He also found that the lower the temperature at which the determination was carried out the greater the deviation. Albumin solutions deviated

at the lower rates of shear, while the law seemed to hold with casein sols.

Starch sols were prepared by heating both the soluble and insoluble modifications. Poiseuille's law was found to hold with the soluble starch but not with the insoluble starch, neither did it hold with agar-agar or soap solutions. Rothlin concludes that Poiseuille's law does not hold with those sols which have a tendency toward gel formation, and explains that in such sols a cohesive force must be overcome.

Rothmann (1914), in his investigations on the viscosity of blood, found that for frogs' blood Poiseuille's law held for a capillary tube having a diameter of 465μ, but it did not hold for a tube whose diameter was 100μ. He believed that the validity of Poiseuille's law depended on the ratio of particle size to capillary diameter, the larger the value of this ratio the greater the deviation from the law.

Humphrey and Hatschek (1916), using a modified Couette apparatus, found that the viscosity of rigid particles was a function of the rate of shear.

Shorter (1906) (1909) found that saponin, peptone, and albumin solutions affected the viscosity of the surface of their solution, so that a definite change in viscosity could be detected when a complete layer of molecules was formed on the surface. Dienes (1911), using a Coulomb viscosimeter, found the viscosity of water and salt solutions increased with the depth of immersion of the disc; while with sols of egg white, horse blood serum, and methyl violet the greatest viscosity was obtained just beneath the surface, the viscosity at first decreasing with increasing depth of immersion until a minimum value was reached, and then increasing. Gurney (1908b), using a modified Couette apparatus, found that water exhibited considerable rigidity of surface, amounting to four or five times its viscosity at extremely low rates of shear.

Odén (1912), in his investigations on the properties of very concentrated sulphur hydrosols, measured the viscosity of the sols of various concentrations. He found that the sols whose particles were in a finer state of subdivision had the greatest viscosity. The difference was more marked at the higher concentrations.

Schroeder (1903), Ostwald (1905) and others have found that prolonged boiling decreases the viscosity of gelatin solutions. Some of the factors influencing the viscosity of gelatin and glues have recently been investigated by Bogue (1920) (1921).

Woudstra (1908) carried out an investigation of the viscosity of suspensoids. He (1911) found that the coagulation of iron oxide sols could be followed by measuring the viscosity of the solution. A decrease in viscosity occurred which he attributed to a decrease in the

degree of dispersion. This decrease was followed by an increase in viscosity due to the large particles coagulating out and stopping up the capillary. Freundlich and Ishizaka (1913) investigated the change in the viscosity of aluminum hydroxide sols with time, after the addition of a coagulating agent. They found that the viscosity did not change very much at first, but later increased rather rapidly. They explain this failure of the sol to show an immediate increase in viscosity as due to time being required for the adsorption of the coagulating agent.

Chick and Martin (1912), Chick and Lubrzynska (1914), and Chick (1914) used the viscosity of protein solutions as a means for the determination of the hydration of proteins in solution. They found by applying Hatschek's (1911) equation that as much as 8.6 cc. of water was taken up by one gram of casein. Arrhenius (1916) took exception to such high hydration values and by applying his own equation obtained much lower figures.

Effect of electrolytes on viscosity of emulsoids.—Laqueur and Sackur (1903) in their study of casein found that the addition of acid and alkali to the casein sols affected their viscosity. They attributed this effect to a change in the ionization of the casein. They found that the viscosity passed through a maximum with increasing hydroxyl-ion concentration. Schroeder (1903) investigated the effect of hydrochloric acid on the viscosity of gelatin. Pauli and Handovsky (1909), and Handovsky (1910) studied the effect of the addition of various amounts of different acids and alkalies on the viscosity of albumin. These authors found that the different acids, when compared on the basis of normality, affected the viscosity in various ways. Sulfuric acid caused only a slight increase, as did acetic acid; trichlor acetic acid caused a slight increase and then a fall; dichlor acetic acid caused a considerable rise and then a fall; while monochlor acetic acid caused a very steep rise over the entire range. Citric acid caused a steady rise over the entire range; oxalic acid caused a rise and then a fall; while hydrochloric acid caused a very great rise and then a very sharp fall in viscosity as the concentration of acid increased.

The effect of alkalies was found to be related more or less to their dissociation constants, the greater the dissociation constants the greater the increase in viscosity produced. There was no decrease in viscosity at the higher concentrations. Their investigation was limited mainly to the monovalent bases. The highest concentrations investigated in the case of both acids and bases was 0.05 normal.

Michaelis and Mostynski (1910) studied the effect of acid and alkali on serum from which the globulin had been separated by prolonged dialysis. They found that the addition of acid at first caused

a decrease in viscosity in the region of a hydrogen-ion concentration of $0.28-2.0\times10^{-5}$, and then an increase with increasing acidity. This decrease was not mentioned by Pauli and Handovsky (1909). The addition of alkali to completely dialyzed serum always caused an increase in viscosity.

Pauli (1913) found that on the addition of hydrochloric acid to albumin the maximum viscosity was reached at the point where the value for the difference between bound hydrogen and bound chloride ions was the greatest. He also pointed out that by the addition of increasing amounts of hydrochloric acid to gelatin, the osmotic pressure at first increased to a maximum and then decreased. Expressing the results graphically, the curve for osmotic pressure was very similar in form to the viscosity curve produced under the same conditions.

In recent years numerous investigators have studied the effect of electrolytes on the viscosity of protein sols. Noteworthy among these is Loeb (1920a) (1920b) (1920c) (1921a), who presented evidence tending to show that the maximum viscosity with the various acids was reached at the same hydrogen-ion concentration, and that for a given protein the same high point was reached with the various acids, with the exception of sulfuric acid, which always produced a smaller increase in viscosity than the other acids. The same results in general were found to hold true with the alkalies. The monovalent bases reached the same maximum at the same hydroxyl-ion concentration. The divalent bases differed in that they did not cause such a great increase in viscosity. Loeb explained the behavior of salts in decreasing the effect of acids on viscosity as due to the anion alone, while the cation was thought to be the effective ion in the case of the alkalies. Loeb (1921b) (1921c) (1921d) (1921e) (1921f) agrees with the explanation of imbibition as put forward by Proctor (1914), Proctor and Wilson (1916), and Wilson and Wilson (1918), based on the Donnan (1911) equilibrium as established by Donnan and Harris (1911), Donnan and Allmand (1914), and Donnan and Garner (1919).

Zoller (1919) (1920) (1921) studied the effect of different hydrogen-ion concentrations on the viscosity of casein solutions and found the maximum viscosity occurred at a pH of 9.

Ostwald (1913) points out that while the viscosity of a molecular solution is defined by the concentration and the temperature, another factor enters into the definition of the viscosity of a suspension, namely, the degree of dispersion. In the case of emulsoids, Ostwald (1913) gives ten factors which may influence viscosity, namely, (1) concentration, (2) temperature, (3) degree of dispersion, (4) solvate formation, (5) electric charge or ionization, (6) previous thermal treatment, (7) previous mechanical treatment, (8) inoculation with

small quantities of more viscous colloids, (9) time, and (10) addition of both electrolytes and non-electrolytes. Another factor which is of extreme importance should be added to this list, namely, the rate of shear.

Practical applications of the viscosity of colloids.—The viscosity of colloidal solutions of rubber, soluble cellulose compounds, starch, soap, paints, clays, glues, gelatin, etc., has been investigated by numerous workers. With certain limitations it has been found that the viscosity determination could be made to serve as an index to quality.

In biology and medicine certain changes in the animal organism have been found to manifest themselves by changes in the viscosity of the body fluids such as the blood, milk, etc.

EXPERIMENTAL

THE PROBLEM

The object of this work was to continue the investigations of flour strength along the lines of colloid chemistry, (1) by studying the use of viscosity measurements as a means of determining the imbibitional power of the gluten proteins without their removal from the flour; (2) to see what correlation, if any, could be derived between the viscosity measurements and the baking strength of the flour.

In the work reported here no attempt was made to investigate all the factors which might influence the viscosity of flour-in-water suspensions but rather to keep as many factors constant as possible while comparing the behavior of the various flours subjected as nearly as possible to the same conditions. The measurements reported here are more truly a measure of the imbibitional capacity of the flour constituents in a state of coarse suspension as shown by the resistance of their suspensions to a shearing force. In this work no attempt has been made to distinguish between viscosity and plasticity or to determine the plasticity constants for those mixtures which were undoubtedly plastic. The term "viscosity" has been used in a rather loose sense throughout to indicate all resistance offered by the suspensions to the shearing force applied.

FLOURS USED

For the first phase of the work, that is, the effect of various acids on the imbibitional power of the flour as measured by viscosity, two flours were used; B-780, a strong flour milled from northern spring wheat, an especially strong patent flour prepared for a select trade; and B-783, a patent flour milled from soft club wheat grown at Genessee, Idaho. These two flours contained practically the same percentage of crude protein. B-780 had a total protein content

(N×5.70) of 12.13 per cent; the water-soluble protein, determined by digesting for 40 minutes at 10° C., was 1.63 per cent; and the loaf volume, using 300 grams of flour, was 1639 cc. Flour B-783 contained 12.15 per cent of total protein and 1.67 per cent of water-soluble protein, and gave a loaf volume of 1530 cc. Additional analytical and baking data may be found in the paper by Sharp and Gortner (1922). At the beginning of this investigation a considerable quantity of these two flours was placed in air-tight containers and stored in a cold storage plant at a temperature of approximately 3° C.

The second group of flours investigated was kindly supplied by Walter Marshall, of the United States Bureau of Markets. The baking and analytical data were also furnished by him.

The third group of flours was a series collected by the American Institute of Baking from various parts of North America and subjected to a joint investigation by L. A. Rumsey, F. A. Collatz, and Paul F. Sharp, in an effort to investigate as many factors as possible which might be related to flour strength. Rumsey (1922) and Collatz (1922) presented separate reports upon their phases of the investigation.

METHODS AND GENERAL OBSERVATIONS

In beginning the experimental work on the viscosity of flour-in-water suspensions, the method employed by Lüers and Ostwald (1919) was used. A viscosimeter of the Ostwald type was constructed which had a bulb of about 23 cc. capacity. A bulb of this size was chosen in order that 25 cc. of liquid might be used to operate the viscosimeter. The capillary had a length of about 9 centimeters and a diameter of 0.85 millimeters. The time of outflow for water as determined by a stop watch was 9 1/5 seconds. The viscosity determinations were all carried out in a glass-walled thermostat at a temperature of 25° C.

As Lüers and Ostwald (1919) used a 20 per cent flour-in-water suspension for their investigations of the effect of lactic acid on the viscosity of wheat flours, this same concentration was chosen for the first part of the investigation.

In the first place it was noticed that successive readings on the same 20 per cent flour-water mixture without the addition of acid gradually decreased. This behavior was observed more or less with all the flours investigated. As an example of this behavior some of the data obtained with flour No. 2116, milled from No. 3 dark northern spring wheat and producing a loaf of large volume, are given. The determinations were made one after the other as fast as possible. After each reading, air was blown through the mixture in the viscosimeter to stir it thoroly, and the next reading was taken immediately after-

ward. It was at first thought that this decrease in viscosity might be due to the effect of carbon dioxide of the air, so in several instances the air used in stirring the mixture was first passed through a solution of sodium hydroxide. However, this procedure did not in the least affect the results.

One set of results obtained with this flour, expressing the rate of outflow in fifths of seconds, the first reading being taken 15 minutes after preparation of the flour-in-water, is as follows: 329, 324, 329, 314, 304, 302, 303, 289, 283, 283, 281, 281, 270, 262, 260, 250, 252, 242, 242, 236, 236, 236, 230, 229, 225, 213, 219, 213, 214, 217, 212. From other observations it was believed that this effect was not due to time of standing. This point was investigated by preparing mixtures of flour and water, determining their viscosity, and then letting them stand for an hour or more. Results obtained with the same flour are presented as an example of the effect of standing. The time of outflow observed 15 minutes after the preparation of the mixture was 341 and a reading immediately afterwards gave 346. The material was then allowed to stand for an hour in the viscosimeter. It was then mixed by blowing air through the apparatus and the reading obtained was 346 and the subsequent reading was 341.

It was thus very difficult to decide which reading was the correct one. It was thought that perhaps the difficulty might be due to the method of preparation of the suspension, therefore three different methods were tried: (1) the weighed amount of flour was mixed with the required amount of water by suddenly shaking; (2) the weighed amount of flour was placed in a beaker and water was added slowly from a burette, the flour being stirred in the meantime with a stirring rod; (3) enough water was first added to the weighed amount of flour in a mortar to make a dough, and this dough was kneaded with the pestle for about three minutes and then the rest of the water was gradually worked in with the dough, forming the suspension.

The results obtained with the three methods seem to indicate that the third method gives the most concordant results with the original suspension before acid was added. Serious difficulties were encountered by the use of Methods 2 and 3 when adding the acid to mixtures obtained in this way, for after the addition of a given amount of acid the viscosity gradually increased until after a time a maximum was reached and from then on the viscosity declined. In one instance it was only after the twenty-ninth reading that the maximum viscosity was reached, the readings increasing from 462 fifths of seconds to 1003 fifths of seconds. In the first method this behavior was not so marked, the maximum being reached after the second and third readings. This was believed to be due to the fact that in the first method

the gluten particles were mechanically separated to a greater extent, thus presenting a greater surface upon which the acid might act; while in the other cases, in spite of the care taken to prevent it, small particles of gluten were present and time was required for the acid to diffuse to their center, thus causing maximum swelling.

Another difficulty encountered was the effect of air bubbles produced by the shaking, in the first method, or by the air that was blown through the apparatus to stir the mixture. In an attempt to prevent the formation of air bubbles it was necessary in many cases to remove the viscosimeter from the bath and invert it carefully several times in order to accomplish the mixing.

The difficulties encountered indicated that a definite mechanical procedure must be adopted if interpretable results were to be obtained, also that the time factor must be kept constant. The greatest objection to the Ostwald type of viscosimeter was found in attempting to keep the time factor constant. In the investigation of the effect of additions of various amounts of normal lactic acid to the 20 per cent flour-in-water suspensions, the time of outflow for the flours investigated was found to range from 41 seconds to more than 8 minutes. The attempt was made to run at least four determinations for each addition of acid. The effect of various concentrations of lactic acid on twelve different flours was investigated, but the results were so erratic that it was thought not worth while to present the data here, and the use of the Ostwald viscosimeter was accordingly abandoned.

It was thought that perhaps a viscosimeter of the torsion balance type might eliminate some of the difficulties encountered with the Ostwald viscosimeter. A MacMichael (1915) viscosimeter of the old type was used. It was soon realized that comparative results were all that could be obtained without the unnecessary expenditure of a great amount of time. The experiments were all carried out at 25° C. The determinations were performed in a constant temperature room. The flour and water were always allowed to come to the temperature of the room before the suspensions were made. In this first set of determinations, enough flour was taken to make 25 grams on the moisture-free basis. To this weight of flour enough water was added to make a total of 100 grams of water. The actual amount of water added was thus approximately 96.5 grams. This mixture of flour and water was allowed to stand one hour with occasional shaking. This interval of time was chosen because Bailey and Collatz (1921) had found that the quantity of electrolytes extracted from flour by water at first increased rapidly with time of extraction but that later the amount extracted became nearly constant. It was therefore believed that an easily duplicable level of electrolyte content could be obtained at the end of one

hour and that the proteolytic action of enzymes on the gluten would be inappreciable during this short period. A departure from the general method for using viscosimeters of this type was adopted because comparative results are all that were expected, and the attempt was made to make the method as rapid as possible. Instead of waiting for the reading on the dial to become constant, the motor in the instrument was started and the first high rotation of the dial was read. The motor was then stopped, the material in the cup was stirred several times with the plunger while the cup was rotated with the hand, and another reading was immediately taken. This procedure permitted duplicate readings to be taken very rapidly and did not introduce appreciable errors due to settling out of the suspension. The duplicate readings obtained by this method always checked as close as the dial could be read accurately. The results of the determinations carried out by this method are all reported in terms of angular rotation in degrees MacMichael, a reading of 300 degrees MacMichael indicating that the dial has made one complete revolution. The wires were tested frequently to see if the limit of their elasticity had been exceeded. The readings were not converted over to absolute viscosity units because doubts were entertained as to the true value of the results expressed in absolute units.

The results would seem to indicate that the viscosity in some of the flour suspensions, at least, varied with the rate of shear and that even the different flours vary in this respect. Most of the shear probably came between the bottom of the disc and the cup where the rate of shear increased from zero at the point of common axis to probably its maximum value near the outer under edge of the disc, thus the material was not all subjected to the same rate of shear.

In order to have some idea as to the absolute viscosity values, the instrument was always standardized against sucrose solutions. Owing to its inertia the liquid did not attain its maximum speed at the time the reading was taken, and as a volume of 100 cc. was used in each case, any difference in density between two liquids would affect the viscosity readings. In this first series of experiments the wire was standardized against a sucrose solution whose specific gravity compared with water at 25° C. was 1.185 and whose absolute viscosity standardized by means of water in an Ostwald viscosimeter was found to be 5.73 centipoise. The speed of the motor was so adjusted that the reading obtained with this sucrose solution was as nearly as possible 14.3 degrees, MacMichael. The specific gravity of the flour-in-water suspension compared with water at 25° C. was determined in two instances and was found to be approximately 1.086.

VISCOSITY OF THE ORIGINAL FLOUR-IN-WATER SUSPENSION AS AFFECTED BY VARIOUS AMOUNTS OF DIFFERENT ACIDS

The strength of the various normal acid solutions was determined by titrating aliquots with 0.10 normal sodium hydroxide, using phenolphthalein as an indicator.

In this first series of experiments the effect of the addition of different amounts of normal solutions of various acids on the viscosity of flours B-780 and B-783 was studied. For each determination 100 cc. of the suspension prepared as previously described was placed in the cup of the viscosimeter and four separate readings were made. A definite amount of a normal solution of the acid being investigated was then added. A 1-cc. burette graduated to 0.01 cc. was used to measure the acid. After each addition of acid the mixture was stirred and four separate readings were taken. The additions of acid were continued until a total of 10 cc. had been added.

The effect on viscosity of this dilution with 10 cc. was studied by taking mixtures of various readings ranging from 17 to 230 and adding 10 cc. of water. In all, forty such determinations were made and the average taken. The dilution was found to cause a decrease in the viscosity of 22.9 per cent, the maximum deviation from this average was found to be 1.9 per cent. The effect of dilution was the same with the two flours within experimental error. The assumption was made that the effect of dilution on the viscosity was linear and the results given in the tables have been corrected for dilution on that basis.

The effect on the viscosity of the addition of the following total amounts of normal acid was determined: 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1, 1.20, 1.50, 2, 2.50, 3, 4, 5, 6, 8, and 10 cc. With flour B-783 there was practically no change in viscosity until a total of 1 cc. of acid had been added, while with flour B-780 the increase began with the addition of a lesser amount. It was thought not worth while to record all the results obtained with the smaller additions of acid, so these were omitted in Table III, but in order to show the onset of the increase in viscosity the effect produced by the addition of the smaller amounts of a few of the acids is given in Table II.

TABLE II

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL ACID SOLUTIONS

Twenty-five grams of flour on the dry basis was treated with enough distilled water to make 100 cubic centimeters of water and digested with occasional shaking at 25° C. for one hour. One hundred cubic centimeters of this mixture was taken for the viscosity measurements. All determinations were carried out at the temperature of 25° C., and were corrected for dilution back to the original volume of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. acid added	Hydrochloric acid		Lactic acid		Acetic acid	
	B-780	B-783	B-780	B-783	B-780	B-783
0.00	63	39	64	36	63	37
0.10	66	41	65	37	62	37
0.20	68	42	67	38	63	37
0.30	69	42	71	38	63	37
0.40	76	41	82	38	64	37
0.50	88	40	94	38	69	37
0.60	99	40	110	39	72	37
0.70	120	41	124	41	78	36
0.80	142	43	143	44	82	36
0.90	165	48	157	47	88	36
1.00	187	54	172	52	91	36

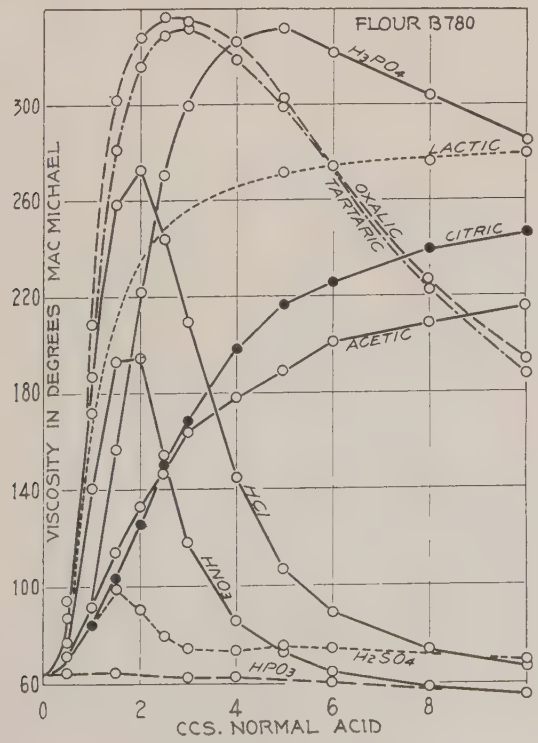

Fig. 1. Viscosity of 100 cc. of 20% Flour-in-Water Suspensions of Strong Flour B-780 as Affected by Different Amounts of Various Acids (Compare with Fig. 2.)

In Table III and Figures 1 and 2 the results obtained with the larger amounts of acid are given. All the values reported in the following tables are the average of the determinations carried out on at least two separate flour suspensions. In order to show the accuracy of these duplicate determinations a few samples have been grouped together and are given in Table IV.

TABLE III

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL ACID SOLUTIONS, WITH PH OF THE RESULTING MIXTURE

Twenty-five grams of flour on the dry basis was taken in each instance, treated with 100 cubic centimeters of distilled water, and digested with occasional shaking at 25° C. for one hour. One hundred cubic centimeters of this mixture was taken for the viscosity measurement. All determinations were carried out at the temperature of 25° C., and were corrected for dilution back to the original volume of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. acid added	Hydrochloric acid				Lactic acid			
	B-780		B-783		B-780		B-783	
	Visc	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	63	5.84	39	5.87	64	5.84	36	5.87
0.50	88	4.75	40	4.95	94	4.84	38	4.90
1.00	187	3.92	54	4.25	172	4.14	52	4.41
1.50	258	3.32	104	3.64	215	3.91	82	4.07
2.00	273	2.65	128	3.12	235	3.75	105	3.88
2.50	244	2.27	115	2.57	248	3.55	119	3.74
3.00	209	1.96	93	2.31	255	3.46	125	3.65
4.00	145	1.79	56	1.89	265	3.32	132	3.49
5.00	107	1.60	41	1.69	272	3.23	136	3.39
6.00	89	1.39	34	1.55	272	...	136	3.28
8.00	73	1.23	29	1.37	277	3.02	139	3.14
10.00	66	1.17	25	1.22	280	2.95	140	3.04

cc. of N. acid added	Tartaric acid				Oxalic acid			
	B-780		B-783		B-780		B-783	
	Visc	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	66	5.86	36	5.90	65	5.83	36	5.82
0.50	83	4.67	37	5.07	87	4.67	36	4.99
1.00	179	4.11	40	4.41	208	4.15	35	4.32
1.50	281	3.67	56	3.97	302	3.71	48	3.90
2.00	317	3.36	84	3.67	328	3.43	71	3.58
2.50	330	3.12	104	3.42	337	3.11	95	3.35
3.00	332	2.83	113	3.21	335	2.94	108	3.09
4.00	320	2.52	118	2.86	326	2.53	113	2.73
5.00	301	2.26	106	2.50	302	2.28	105	2.42
6.00	272	2.06	94	2.25	274	2.12	93	2.15
8.00	223	1.87	72	2.01	227	1.91	72	1.92
10.00	188	1.72	54	1.84	194	1.78	54	1.78

TABLE III—*Continued*

cc. of N. acid added	Sulfuric acid				Acetic acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	66	5.91	38	5.85	63	5.95	37	5.83
0.50	67	4.76	38	...	69	5.08	37	5.16
1.00	86	4.02	33	...	91	4.71	36	4.82
1.50	98	3.41	33	3.62	114	4.55	41	4.60
2.00	90	2.86	33	...	133	4.43	46	4.46
2.50	79	2.42	33	...	147	4.29	50	4.40
3.00	74	2.19	33	2.40	163	4.23	54	4.31
4.00	73	1.91	31	...	178	4.11	63	4.18
5.00	74	1.75	31	...	189	4.01	74	4.07
6.00	74	1.62	30	...	201	...	83	4.01
8.00	72	1.45	28	...	209	3.83	92	3.88
10.00	69	1.36	27	1.39	216	3.76	105	3.79

cc. of N. acid added	Citric acid				Ortho-phosphoric acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	65	5.96	37	5.82	66	5.90	37	5.85
0.50	68	4.97	36	5.16	69	5.24	39	5.43
1.00	83	4.60	36	4.72	94	4.73	38	4.97
1.50	103	4.29	35	4.46	156	4.25	48	4.62
2.00	126	4.07	38	4.25	222	3.86	72	4.28
2.50	150	3.88	43	4.07	270	3.62	110	3.98
3.00	168	3.76	50	3.93	299	3.32	138	3.65
4.00	199	3.55	68	3.60	326	2.95	157	3.25
5.00	217	3.37	81	3.49	332	2.66	163	2.91
6.00	226	3.26	91	3.35	322	2.47	155	2.47
8.00	240	3.09	99	3.18	304	2.26	146	2.38
10.00	247	2.95	103	3.03	285	2.14	136	2.22

cc. of N. acid added	Nitric acid				Meta-phosphoric acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	64	5.96	35	5.85	63	5.90	34	5.85
0.50	77	4.85	34	5.06	63	...	33	...
1.00	140	3.99	39	4.48	62	...	32	...
1.50	193	3.33	57	3.60	62	...	...	...
2.00	195	2.82	66	3.08	61	...	31	...
2.50	154	2.35	55	2.63	...	...	...	...
3.00	118	2.15	43	2.27	62	...	...	...
4.00	85	1.87	33	1.79	62	...	...	...
5.00	73	...	28	1.69	...	...	27	...
6.00	64	1.54	27	...	60	...	...	...
8.00	58	1.36	26	1.35	...	...	...	...
10.00	55	1.23	23	1.23	55	2.26	26	2.33

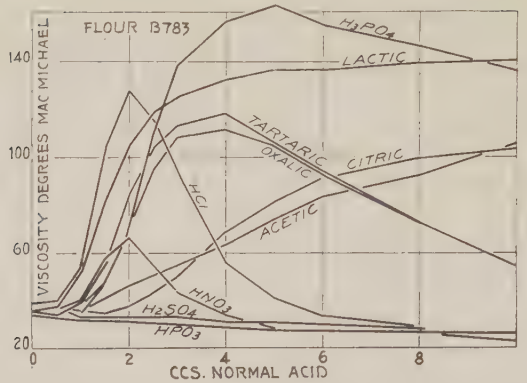

Fig. 2. Viscosity of 100 cc. of 20% Flour-in-Water Suspensions of Weak Flour B-783 as Affected by Different Amounts of Various Acids (Compare with Fig. 1.)

TABLE IV

Viscosity, in Degrees MacMichael, of Flours B-780 and B-783 as Influenced by the Addition of Various Amounts of Normal Lactic and Acetic Acid Solutions Showing Agreement of Duplicate (A and B) Determinations

Twenty-five grams of flour on the dry basis was taken in each instance, treated with enough water to make 100 cubic centimeters and digested with occasional shaking at 25° C. for one hour. One hundred cubic centimeters of this mixture was taken for the viscosity measurements. All determinations were carried out at the temperature of 25° C. and are corrected for dilution back to the original volume of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. acid added	Lactic acid				Acetic acid			
	B-780		B-783		B-780		B-783	
	Visc.	Visc.	Visc.	Visc.	Visc.	Visc.	Visc.	Visc.
	A	B	A	B	A	B	A	B
0.00	64	64	36	37	65	61	37	37
0.50	94	94	39	38	70	68	37	37
1.00	174	170	50	53	91	90	35	36
1.50	218	212	80	84	116	112	41	41
2.00	237	233	105	105	135	131	46	46
2.50	250	246	119	119	147	147	50	50
3.00	254	255	125	124	166	160	54	54
4.00	264	265	135	129	179	177	64	62
5.00	272	272	139	133	190	188	73	74
6.00	272	271	139	133	202	199	82	83
8.00	276	277	143	135	209	209	92	91
10.00	280	280	144	136	215	217	107	103

In Table V are given the results obtained with phosphoric, monochlor acetic, and dichlor acetic acids. In this case a new adjustment of the wire in the viscosimeter was made. For standardization a 60 per cent sucrose solution of a density at 25° C. of 1.284 and whose absolute viscosity was 43.86 centipoise was used [Bingham and Jackson (1917)], the speed of the motor and the distance of the plunger from the bottom of the cup were so adjusted as to make the reading as nearly

as possible 87.7 degrees MacMichael. It was feared that the results obtained with this readjusted wire would not be the same as those obtained with it in the previous condition. The effect of lactic acid on the viscosity of the two flours was therefore redetermined in order to give a basis for comparison with the preceding series. It will be noted that the first reading taken with flour B-783 is practically the same in both series, but with B-780 the original reading is much higher in the second series than in the first. This difference between the two flours holds more or less when the effects of the different additions of lactic acid are compared.

TABLE V

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL ACID SOLUTIONS, WITH THE pH OF RESULTING MIXTURE

Twenty-five grams of flour on the dry basis was taken in each instance, treated with 100 cubic centimeters of distilled water and digested with occasional shaking at 25° C. for one hour. One hundred cubic centimeters of this mixture was taken for the viscosity measurements. All determinations were carried out at the temperature of 25° C., and are corrected for dilution back to the original volume of 100 cubic centimeters. The wire in this case was standardized with a 60 per cent sucrose solution whose absolute viscosity is 43.86 centipoise. This solution gave a reading of 87.7 degrees MacMichael.

cc. of N. acid added	Lactic acid				Phosphoric acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	79	5.84	35	5.87	80	5.85	35	5.83
0.50	127	4.84	40	4.90	90	5.16	38	5.07
1.00	216	4.14	70	4.41	118	4.60	40	4.85
1.50	259	3.91	102	4.07	180	4.09	47	4.36
2.00	280	3.75	122	3.88	246	3.75	64	4.14
2.50	293	3.55	131	3.74	290	3.18	94	3.82
3.00	297	3.46	138	3.65	315	3.03	123	3.62
4.00	299	3.32	143	3.49	332	2.82	156	3.13
5.00	302	3.23	144	3.39	329	2.47	160	2.76
6.00	301	...	143	3.28	314	2.13	156	2.49
8.00	306	3.02	146	3.14	285	2.07	146	2.18
10.00	308	2.95	147	3.04	257	1.96	129	1.94

cc. of N. acid added	Monochlor acetic acid				Trichlor acetic acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	79	5.85	35	5.80	81	...	33	...
0.50	110	4.70	39	4.82	89	...	33	...
1.00	206	3.94	52	4.13	120	...	36	...
1.50	275	3.58	89	3.74	147	...	39	...
2.00	303	3.26	120	3.38	135	...	41	...
2.50	311	3.04	128	3.21	115	...	37	...
3.00	311	2.91	129	3.03	104	...	35	...
4.00	295	2.74	130	2.81	96	...	31	...
5.00	284	2.59	126	2.69	91	...	31	...
6.00	271	2.49	118	2.57	84	...	31	...
8.00	254	2.35	115	2.42	82	...	31	...
10.00	240	2.23	106	2.32	77	...	29	...

EFFECT OF ADDITION OF VARIOUS AMOUNTS OF NORMAL SOLUTIONS OF VARIOUS ACIDS ON HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentration was determined only when there was an appreciable change in the viscosity of the mixture. For each determination of hydrogen-ion concentration, 20 cc. of the 20 per cent flour-water mixture was placed in a test tube and a proportional amount of normal acid was added to correspond to the additions as shown in Tables III and V. The test tube was shaken at intervals during half an hour and then the hydrogen-ion concentration was measured. A Leeds and Northrup potentiometer was used for the measurement of the voltage. A type R high sensitivity galvanometer was used as a current detector, and the hydrogen electrodes were of the Bailey (1920) type, so modified as to contain a larger volume of liquid. All determinations were made in a constant temperature room kept at 25° C. A normal KCl calomel electrode and a flowing junction of saturated KCl were used. The millivolt readings were changed to pH by means of the table of Schmidt and Hoagland (1919). The viscosity readings as in Table III as ordinates and the pH values as abscissa are expressed graphically in Figures 3 and 4.

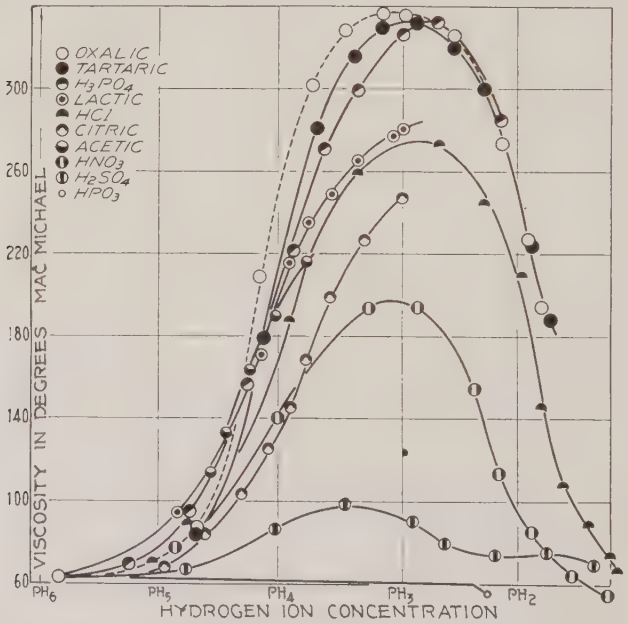

Fig. 3. Viscosity of 100 cc. of 20% Flour-in-Water Suspensions of Strong Flour B-780 at Different Hydrogen-ion Concentrations Produced by Various Acids
(Compare with Figs. 1 and 4.)

An attempt was made to measure the hydrogen-ion concentration in the case of the additions of trichlor acetic acid, but constant readings could not be obtained, especially of the higher additions of acid. It was suspected that the trichlor acetic acid was being reduced on the platinum electrode and that hydrochloric acid was being liberated. In order partially to test this hypothesis, a bubbling electrode of the Hildebrand (1913) type was placed in a 0.10 normal solution of trichlor acetic acid and hydrogen was permitted to bubble around the electrode for several hours. At the end of this time the liquid gave a heavy precipitate with silver nitrate. The original solution gave no precipitate. It was later found that dichlor acetic acid behaved in a somewhat similar way, altho not to such a marked extent. No trouble was encountered in the use of monochlor acetic acid with the hydrogen electrode. This would seem to indicate that the first chlorine atom is split off rather rapidly from trichlor acetic acid, the second less rapidly, and the third not at all or only at a very slow rate.

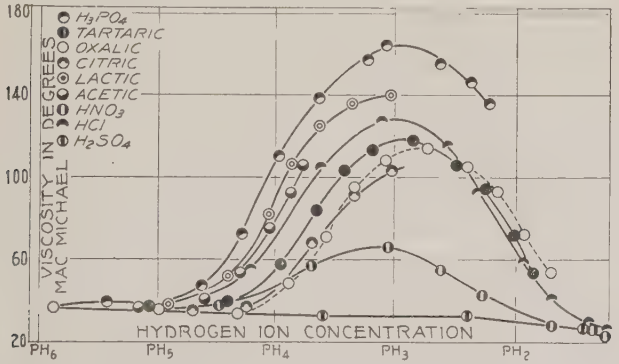

Fig. 4. Viscosity of 100 cc. of 20% Flour-in-Water Suspensions of Weak Flour B-783 at Different Hydrogen-ion Concentrations Produced by Various Acids (Compare with Figs. 2 and 3.)

EFFECT OF ADDED SALTS ON IMBIBITION PRODUCED BY LACTIC ACID

The water with which the flour was digested was made 0.01 normal with respect to various salts with the exception of dibasic potassium phosphate, of which a solution of 0.005 molar was used. The effect of these salts on imbition produced by lactic acid was measured.

In preparing the suspension, 25 grams of flour on the dry basis was treated with enough water and various salts to make 100 cc. of 0.01 normal or 0.005 molar salt solution. The mixture was digested for one hour with occasional shaking. The data obtained on the addition of various amounts of lactic acid to the flour suspension, using flour B-780,

are given in Table VI. The results obtained with flour B-783 are found in Table VII. The data of Tables VI and VII are shown graphically in Figure 5.

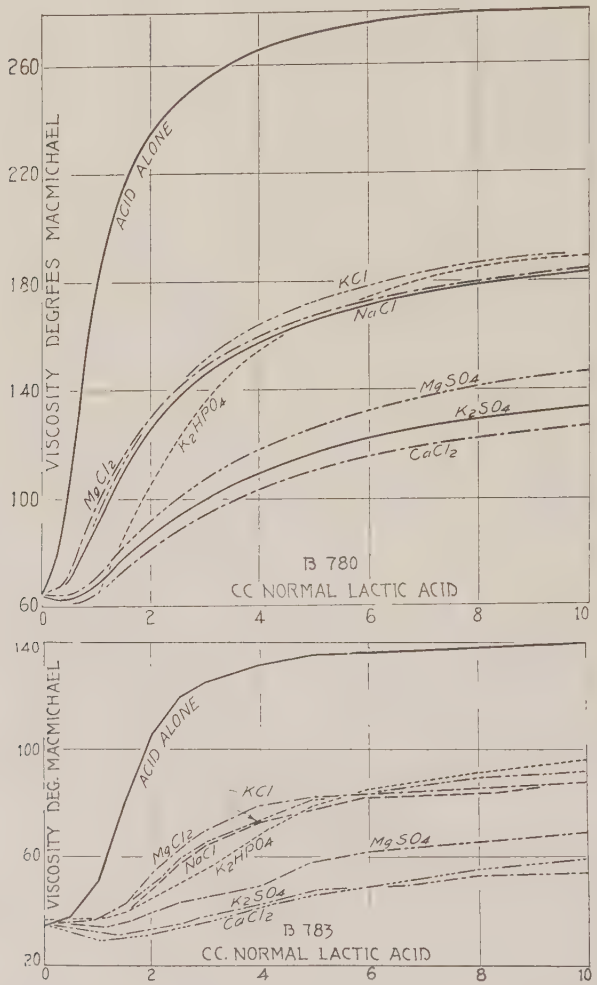

Fig. 5. Viscosity of 100 cc. of 20% Flour-in-Water Suspensions of B-780 and B-783 Made 0.01 Normal with Respect to Various Salts and with the Subsequent Addition of Various Amounts of Lactic Acid

TABLE VI

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOUR B-780 AS INFLUENCED BY VARIOUS AMOUNTS OF NORMAL LACTIC ACID IN THE PRESENCE OF 0.01 NORMAL SOLUTIONS OF VARIOUS SALTS

Twenty-five grams of flour on the dry basis was taken in each instance and treated with enough salt and water to make 100 cubic centimeters of 0.01 normal salt solution. After one hour digestion with occasional shaking, 100 cubic centimeters of this mixture was taken for viscosity measurements. All determinations were made at 25° C., and are corrected for dilution back to the original value of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. acid added	Lactic acid alone	0.005 M. K_2HPO_4	0.01 N. $MgCl_2$	0.01 N. $MgSO_4$	0.01 N. K_2SO_4	0.01 N. KCl	0.01 N. NaCl	0.01 N. $CaCl_2$
0.00	64	64	63	64	65	65	63	63
0.50	94	61	68	64	62	70	69	61
1.00	172	62	95	70	68	90	89	63
1.50	215	85	115	83	76	114	111	73
2.00	235	107	128	91	87	128	125	82
2.50	248	123	140	101	96	142	137	88
3.00	255	136	147	108	98	151	145	93
4.00	265	155	160	117	109	164	158	103
5.00	272	166	167	126	114	170	166	109
6.00	272	174	173	132	123	176	172	115
8.00	277	185	180	141	129	187	179	121
10.00	280	189	183	146	134	188	183	127

TABLE VII

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOUR B-783 AS INFLUENCED BY VARIOUS AMOUNTS OF NORMAL LACTIC ACID IN THE PRESENCE OF 0.01 NORMAL SOLUTIONS OF VARIOUS SALTS

Twenty-five grams of flour on the dry basis was taken in each instance and treated with enough salt and water to make 100 cubic centimeters of 0.01 normal salt solution. After one hour digestion with occasional shaking, 100 cubic centimeters of this mixture was taken for viscosity measurements. All determinations were made at 25° C. and are corrected for dilution back to the original value of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. acid added	Lactic acid alone	0.005 M. K_2HPO_4	0.01 N. $MgCl_2$	0.01 N. $MgSO_4$	0.01 N. K_2SO_4	0.01 N. KCl	0.01 N. NaCl	0.01 N. $CaCl_2$
0.00	36	37	36	37	37	37	36	35
0.50	38	38	35	36	35	36	35	33
1.00	52	36	37	33	32	35	35	30
1.50	82	40	43	35	31	42	40	30
2.00	105	45	54	40	33	49	49	32
2.50	119	51	63	43	36	58	58	35
3.00	125	56	70	46	39	66	64	36
4.00	132	69	79	50	43	74	72	43
5.00	136	79	82	59	47	81	78	46
6.00	136	85	83	63	49	85	82	49
8.00	139	93	85	66	56	90	83	53
10.00	140	97	87	69	60	92	87	55

EFFECT OF ELECTROLYTES PRESENT IN THE ORIGINAL FLOUR ON VISCOSITY

It is a matter of common knowledge that the addition of various salts to an acid solution decreases the power of the acid to produce imbibition in emulsoid colloids. That the gluten of wheat is no exception to this rule has been shown by Wood (1907b), Wood and Hardy (1908), Upson and Calvin (1916), Gortner and Doherty (1918), and Sharp and Gortner (1922). The presence of a relatively large amount of soluble electrolytes in the water extract of wheat flour was shown by Bailey and Collatz (1921), who, in addition, found that the quantity of electrolytes bore a definite relation to the ash content of the flour. It was believed that the presence of these salts was exerting an inhibiting effect on the power of the acid to produce imbibition in the flour suspensions studied. In order to ascertain whether or not the marked imbibitional power of flour B-780 with the various acids as compared with flour B-783 was due to the difference in ash content and consequently in soluble electrolytes, 25 grams of flour was treated with one liter of water and after standing for one hour with occasional shaking the material was centrifuged, the liquid containing the soluble ash was decanted, and the residue was made up to a total volume of 100 cc. with distilled water. The effect of lactic acid on the viscosity of the mixture was determined. It was noted that imbibition began with the addition of the first 0.10 cc. of normal acid and that the maximum imbibition reached was much higher than when the electrolytes were not first extracted. This indicated clearly that a considerable proportion of the soluble electrolytes had been removed and that the presence of the electrolytes in the experiments reported in Tables II, III, IV, and V was repressing to a considerable extent the power of imbibition of the proteins present. The true imbibitional powers of the proteins were thus masked to a greater or less extent by the effect of the salts present in the water extract. Even with the electrolytes partially removed by this method, flour B-780 still shows a much greater power of imbibition than does flour B-783, altho the differences were not so marked as in the original untreated flour-water mixture. In the experiments in which the water extract has been removed, the viscosities are more nearly in accord with the loaf volume of the flours than in the previous instance. In the study of the effect of the various acids with the water extract removed, the quantity of flour taken was decreased so that the high viscosity readings obtained would not exceed the limits of elasticity of the wire of the viscosimeter.

In order to ascertain whether or not this treatment removed all the electrolytes which were inhibiting imbibition, an investigation was carried out on two flours selected because of their widely different

ash content. Flour 1004 had an ash content of 0.92 per cent on the dry basis and flour No. 1008 had an ash content of 0.52 per cent, thus the ash contents of the two flours were in a ratio of 1 to 1.77.

Experiments were carried out in which 18 grams of flour on the dry basis was treated with enough water to make a total volume of 100 cc. and the mixture was digested with frequent shaking for one hour. At the end of this time the material was centrifuged, the supernatant liquid decanted, and its electrical conductivity measured at 30° C.

A second 18 grams of flour on the dry basis was treated with one liter of distilled water and digested for one hour with occasional shaking. At the end of this time the material was centrifuged and the supernatant liquid discarded. The residue was treated with enough distilled water to make a total volume of 100 cc. and shaken at intervals during 15 minutes, again centrifuged, and the conductivity of the supernatant liquid determined. This procedure was repeated with the exception that after making up to the final volume of 100 cc. the effect of the additions of various amounts of normal lactic acid on the viscosity was determined.

A third set of experiments was performed in which 18 grams of flour on the dry basis was treated with one liter of distilled water and digested with occasional shaking for one hour. The supernatant liquid was removed and the residue again made up to the volume of one liter and digested for half an hour with occasional shaking. The supernatant liquid was then removed and the residue made up to a total volume of 100 cc. and allowed to stand with occasional shaking for 15 minutes. The material was centrifuged and the conductivity of the extract measured. This procedure was repeated except that after making up to the final volume of 100 cc. the effect of the addition of various amounts of normal lactic acid on the viscosity of the mixture was determined. The results of this study are given in Table VIII.

TABLE VIII

EXTENT TO WHICH SOLUBLE ELECTROLYTES ARE REMOVED FROM FLOUR BY EXTRACTION WITH VARIOUS AMOUNTS OF WATER, TOGETHER WITH EFFECT OF SUCH REMOVAL ON MAXIMUM INCREASE IN VISCOSITY PRODUCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL LACTIC ACID

Flour No.	Ash per cent*	Specific conductance at 30°C. of residue made up to 100 cc.			Highest viscosity reached with lactic acid at 25°C.		
		Direct extraction with 100 cc. of water	After 1 extraction with 1 liter of water	After 2 extractions with 1 liter of water	Without previous extraction	After 1 extraction with 1 liter of water	After 2 extractions with 1 liter of water
1004	0.92	0.00172	0.000110	0.000034	75	329	324
1008	0.52	0.00109	0.000071	0.000028	393	502	498

* Ash calculated on dry basis.

An examination of the results shown in Table VIII indicates that in both cases the extraction with the first liter of water decreased the electrolyte content as indicated by the conductivity determinations to less than one-fifteenth of its original value. The extraction with an additional liter of water decreased the electrolyte content of 1004 to less than one-fiftieth of its original value while reducing the electrolyte content of 1008 to less than one thirty-eighth of its original value.

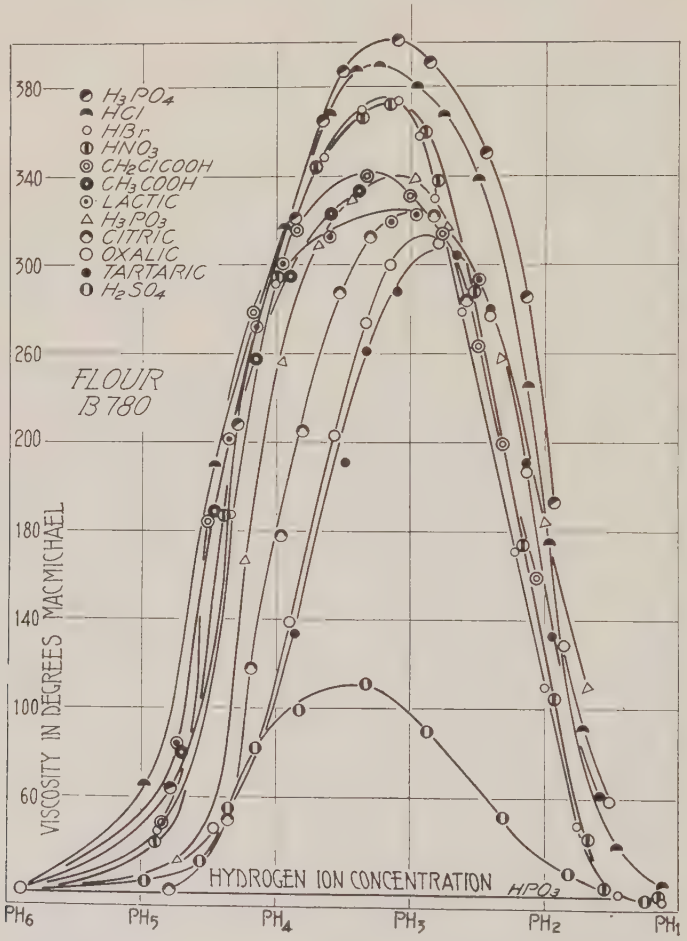

Fig. 6. Viscosity of Extracted Flour, 18 Grams of Extracted B-780 Made Up to 100 cc. and Treated with Different Amounts of Various Acids (Compare with Fig. 7.)

The ratio of the ash contents of the two flours is 1 to 1.77. The ratio of the conductivity of the first extracts is 1 to 1.58, of the second extracts 1 to 1.55, and of the third extracts 1 to 1.21. The results obtained in the viscosity measurements are the same, within experimental error, whether extracted with one liter of water or with two. That is..

the actual content of the electrolytes was so small that changing the ratio from 1 to 1.55 to 1 to 1.21 and decreasing their amounts from 2.5 to 3.2 times seemed to make no appreciable difference in the viscosity readings. These results were therefore taken to indicate that the effect of salts on imbibition could be eliminated by digesting with one liter of water and discarding the extract. Later results seem to indicate that this is not strictly true, but that subsequent extraction may cause some increase in viscosity.

EFFECT OF VARIOUS CONCENTRATIONS OF DIFFERENT ACIDS ON VISCOSITY OF EXTRACTED FLOURS

The test showing the effect of the various acids on viscosity was repeated, using 18 grams of flour on the dry basis, extracting one hour with occasional shaking with one liter of water, centrifuging, discarding the extract, and making the residue up to a total volume of 100 cc. The results are recorded in Table IX and expressed graphically in Figures 6 and 7. The values obtained for the hydrogen-ion concentrations given in Table IX and Figures 6 and 7 were not obtained in the same way as were those given in Tables III and V. The quantity of flour was too small to use the previous method, therefore the expedient

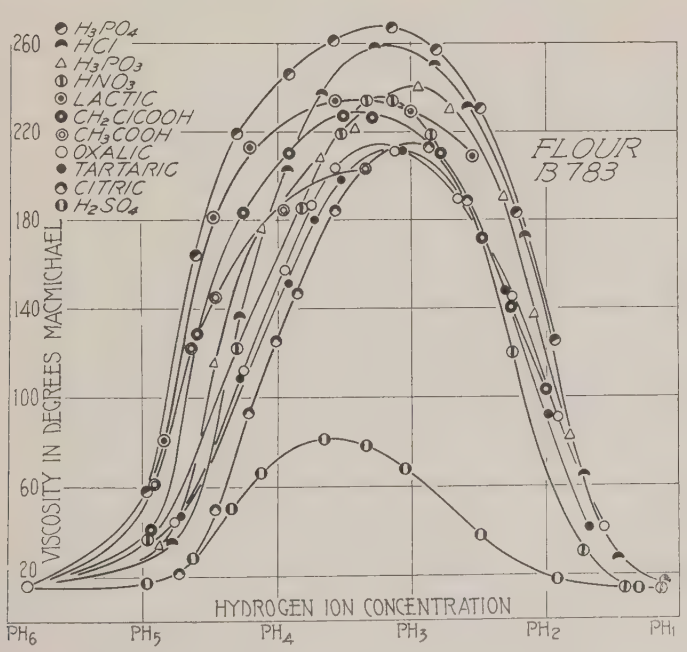

Fig. 7. Viscosity of Extracted Flour, 18 Grams of Extracted B-783 Made Up to 100 cc. and Treated with Different Amounts of Various Acids (Compare with Fig. 6.)

was adopted of using a Hildebrand electrode and measuring the hydrogen-ion concentration after the addition of each amount of acid. For this purpose 1.8 grams of flour on the dry basis was treated with 100 cc. of water with occasional shaking for one hour, centrifuged, the supernatant liquid decanted, and the residue made up to a total volume of 10 cc. A large funnel whose stem had been cut off and closed with a rubber stopper was used for the electrode vessel. This prevented the escape of froth which was constantly broken down with a stirring rod and permitted the use of a small volume of liquid. Instead of using normal acid solutions for the first additions, one-tenth normal solutions were used until a total of one cc. had been added, from then on normal acid was added. As far as could be detected this additional dilution did not affect the hydrogen-ion concentration. The results in this case are not as accurate as in the previous set but are probably correct to within 0.05 pH.

TABLE IX

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY ADDITION OF VARIOUS AMOUNTS OF NORMAL ACID SOLUTION, WITH THE PH OF RESULTING MIXTURE*

The water soluble substances present in the natural flour were largely removed by digesting 18 grams of flour on the dry basis with 1000 cubic centimeters of distilled water for one hour at 25° C., with occasional shaking. The supernatant liquid was removed by centrifuging and the material remaining was made up to a volume of 100 cubic centimeters with distilled water. The viscosity values have all been corrected for dilution back to the original volume of 100 cubic centimeters. All determinations were carried out at the temperature of 25° C. The wire in this case was standardized with a 60 per cent sucrose solution whose absolute viscosity is 43.86 centipoise. This solution gave a reading of 87.7 degrees MacMichael.

cc. of N. acid added	Hydrochloric acid				Nitric acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	20	5.88	18	5.85	20	5.87	18	5.82
0.10	65	4.97	35	4.77	43	4.87	38	4.94
0.20	209	4.45	136	4.29	187	4.38	122	4.31
0.30	318	3.92	202	3.92	295	3.99	185	3.82
0.40	367	3.60	237	3.67	345	3.69	219	3.53
0.50	387	3.40	252	3.50	367	3.35	234	3.35
0.60	389	3.23	258	3.28	373	3.11	234	3.16
0.70	380	2.94	257	3.03	359	2.89	229	2.99
0.80	367	2.74	250	2.82	338	2.79	218	2.86
1.00	338	2.48	231	2.57	288	2.52	192	2.62
1.50	245	2.11	172	2.15	174	2.16	120	2.25
2.00	174	1.97	122	1.94	105	1.93	72	1.96
3.00	91	1.71	64	1.71	42	1.67	30	1.72
5.00	38	1.45	27	1.44	20	1.55	14	1.40
10.00	21	1.12	16	1.10	16	1.15	14	1.13

* Mr. Axel Olsen kindly assisted in making some of the viscosity determinations reported in this table.

TABLE IX—*Continued*

cc. of N. acid added	Oxalic acid				Tartaric acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	18	5.90	18	5.78	17	5.92	18	5 80
0.10	...	...	...	...	...	...	...	...
0.20	46	4.46	45	4.75	46	4.43	45	4.73
0.30	...	...	...	...	...	...	...	...
0.40	139	3.90	112	4.26	134	3.84	109	4.28
0.50	...	...	...	...	...	...	...	...
0.60	223	3.57	157	3.94	211	3.47	151	3.91
0.70	...	...	...	...	...	...	...	...
0.80	274	3.33	186	3.75	261	3.33	180	3.72
1.00	300	3.14	203	3.57	288	3.09	198	3.53
1.50	310	2.77	211	3.11	305	2.64	211	3.09
2.00	277	2.40	189	2.64	278	2.37	189	2.67
3.00	208	2.13	145	2.25	210	2.13	146	2.30
5.00	129	1.86	91	1.91	133	1.94	92	1.98
10.00	59	1.52	41	1.56	61	1.57	41	1.67

cc. of N. acid added	Ortho-phosphoric acid				Acetic acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	20	5.92	17	5.78	16	5.90	15	5.78
0.10	...	...	...	...	...	...	...	...
0.20	64	4.77	58	4.97	80	4.68	61	4.92
0.30	...	...	...	...	...	...	...	...
0.40	228	4.29	164	4.62	187	4.45	122	4.65
0.50	...	...	...	...	...	...	...	...
0.60	320	3.85	219	4.31	226	4.29	145	4.46
0.70	...	...	...	...	...	...	...	...
0.80	365	3.65	246	3.92	...	...	...	...
1.00	387	3.50	261	3.58	258	4.16	165	4.28
1.50	401	3.08	267	3.16	...	...	...	...
2.00	391	2.86	257	2.82	294	3.85	184	3.96
3.00	350	2.43	230	2.49	...	...	...	...
5.00	285	2.13	183	2.22	323	3.57	203	3.60
10.00	193	1.91	125	1.93	333	3.38	203	3.35

cc. of N. acid added	Citric acid				Phosphorous acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	16	5.87	17	5.82	16	5.87	17	5.78
0.10	...	...	...	...	...	...	...	...
0.20	18	4.77	21	4.72	32	4.72	35	4.85
0.30	...	...	...	...	...	...	...	...
0.40	50	4.35	50	4.46	167	4.23	116	4.48
0.50	...	...	...	...	...	...	...	...
0.60	118	4.18	93	4.21	257	3.94	176	4.11
0.70	...	...	...	...	...	...	...	...
0.80	178	3.96	125	4.02	309	3.67	208	3.67
1.00	225	3.80	147	3.85	329	3.43	221	3.42
1.50	288	3.52	184	3.57	339	2.96	240	2.96
2.00	313	3.30	198	3.31	317	2.71	230	2.72
3.00	...	...	...	...	258	2.33	190	2.33
5.00	322	2.82	213	2.87	184	2.01	137	2.08
10.00	285	2.57	189	2.60	110	1.67	82	1.81

TABLE IX—*Continued*

cc. of N. acid added	Monochlor acetic acid				Lactic acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	19	5.82	16	5.83	16	5.85	15	5.78
0.10	48	4.84	41	4.95	83	4.73	81	4.84
0.20	185	4.51	129	4.60	221	4.35	181	4.48
0.30	279	4.16	183	4.26	276	4.13	213	4.21
0.40	317	3.84	210	3.92	300	3.94	226	4.02
0.50	332	3.50	220	3.72	307	....	228	...
0.60	340	3.33	227	3.53	312	3.60	228	3.72
0.70	339	3.23	230	3.42	...	...	...	...
0.80	336	3.14	226	3.30	317	3.50	234	3.57
1.00	331	2.99	219	3.11	317	3.42	231	3.47
1.50	314	2.76	211	2.77	319	...	233	3.25
2.00	295	2.64	190	2.64	319	3.13	231	3.18
3.00	263	2.49	171	2.47	322	2.94	229	3.01
5.00	219	2.32	140	2.28	316	2.74	226	2.79
10.00	159	2.06	103	2.00	293	2.49	208	2.54

cc. of N. acid added	Sulfuric acid				Hydrobromic acid			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	18	5.87	17	5.78	18	5.87	...	...
0.10	22	4.97	17	4.97	45	4.88	...	...
0.20	31	4.56	28	4.63	187	4.35	...	...
0.30	55	4.36	50	4.36	293	4.01	...	...
0.40	82	4.14	66	4.13	348	3.64	...	...
0.50	99	3.82	76	3.89	370	3.36	...	...
0.60	107	3.57	81	3.64	374	3.08	...	...
0.70	111	3.33	79	3.48	358	2.93	...	...
0.80	106	3.18	78	3.35	330	2.81	...	...
1.00	90	2.87	68	3.06	279	2.62	...	...
1.50	52	2.32	38	2.49	171	2.22	...	...
2.00	38	2.10	27	2.20	110	2.00	...	...
3.00	27	1.81	18	1.91	48	1.76	...	...
5.00	...	...	...	...	17	1.45	...	...
10.00	15	1.25	14	1.30	15	1.15	...	...

cc. of N. acid added	Dichlor acetic acid	Trichlor acetic acid	Meta-phosphoric acid	
			Solution stood several months	Fresh solution
	B-780	B-780	B-780	B-780
	Viscosity	Viscosity	Viscosity	Viscosity
0.00	15	18	17	15
0.10	39	22	18	15
0.20	142	89	22	15
0.30	251	179	36	15
0.40	302	244	54	15
0.50	315	273	68	15
0.60	322	282	79	15
0.70	304	276	87	...
0.80	284	258	94	15
1.00	239	214	100	15
1.50	161	95	106	...
2.00	104	41	98	15
3.00	54	21	78	...
5.00	23	23	39	...
10.00	18	23	22	15

It is noted in Table IX and Figures 6 and 7 that the difference between flours B-780 and B-783 is not so marked as before and also that the imbibition of the various acids with a given flour reaches more nearly the same maximum, altho the differences between the two flours are still very great.

EFFECT OF SODIUM AND BARIUM HYDROXIDE ON VISCOSITY OF FLOUR SUSPENSIONS

The effect of sodium and barium hydroxide on the viscosity of these two flours was studied both with the soluble extract present and with the soluble extract partially removed by the method described above. The hydrogen-ion concentrations were determined with the Hildebrand electrode previously described. It is believed that the carbon dioxide of the air did not interfere with the determination of the hydrogen-ion concentration, but was swept away by the constant stream of hydrogen. Experiments in which the total amount of alkali was added all at once and the hydrogen-ion concentration measured immediately, gave practically the same results as those in which the alkali was added in small portions over a longer time. In the experiments with barium hydroxide, a saturated solution was used instead of a normal solution and the results were all calculated on the basis of a normal solution. The results obtained with the alkalies are

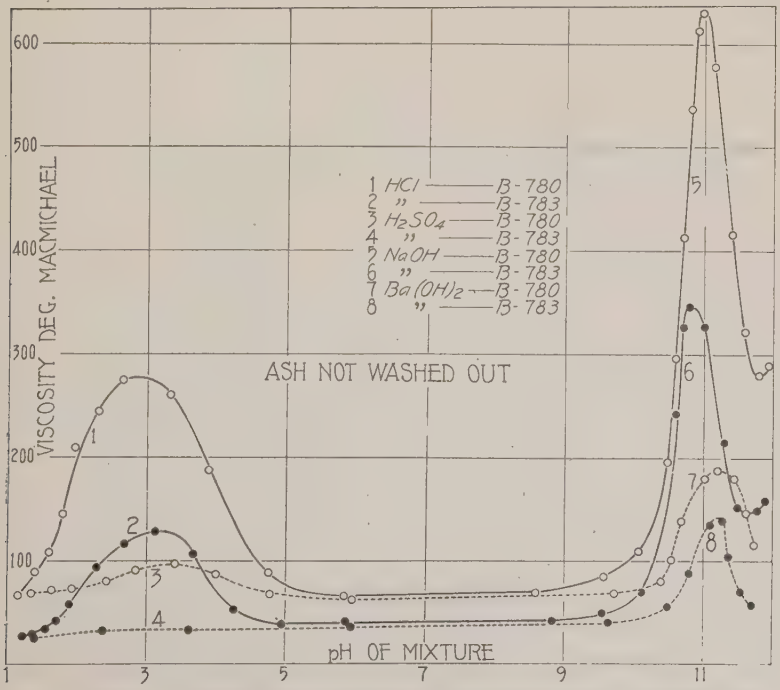

Fig. 8. Relation of Viscosity and Hydrogen-ion Concentration with Unextracted Flour

given in Table X and XI and Figures 8 and 9. For comparison over a range of pH, the effects of hydrochloric and sulfuric acids are also given in the figures.

TABLE X

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL SODIUM AND BARIUM HYDROXIDE SOLUTIONS, WITH pH OF RESULTING MIXTURE*

Twenty-five grams of flour on the dry basis was taken in each instance, treated with 100 cubic centimeters of distilled water, and digested with occasional shaking at 25° C. for one hour. One hundred cubic centimeters of this mixture was taken for the viscosity measurements. All determinations were carried out at the temperature of 25° C., and are corrected for dilution back to the original volume of 100 cubic centimeters. The wire was standardized with a sucrose solution whose absolute viscosity was 5.73 centipoise. This solution gave a reading of 14.3 degrees MacMichael.

cc. of N. alkali added	Sodium hydroxide				Barium hydroxide			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	66	5.85	39	5.85	61	5.92	39	5.82
0.20	66	...	39	...	61	...	...	...
0.40	66	...	39	...	62	...	39	...
0.80	67	8.62	39	...	63	...	40	...
1.00	79	...	40	...	...	...	...	...
1.20	83	9.57	40	8.84	67	...	41	...
1.60	106	10.08	47	9.55	66	9.72	39	...
2.00	194	10.47	66	10.13	76	10.40	40	9.65
2.25	293	10.62	98	...	97	10.55	...	...
2.50	409	10.72	158	...	119	...	51	10.50
2.75	532	10.82	239	10.57	137	10.67	...	...
3.00	609	10.90	321	10.67	152	...	87	10.79
3.25	625	10.97	342	10.79	...	...	...	...
4.00	573	11.16	321	11.02	177	11.02	134	11.12
5.00	411	11.41	211	11.31	185	11.21	140	11.28
6.00	317	11.58	150	11.51	177	11.45	102	11.36
8.00	277	11.78	145	11.80	144	11.58	68	11.56
10.00	287	11.94	155	11.92	114	11.75	55	11.70

* In case of barium hydroxide it was, of course, necessary to use a more dilute solution. The data have all been calculated back to the basis of a normal solution.

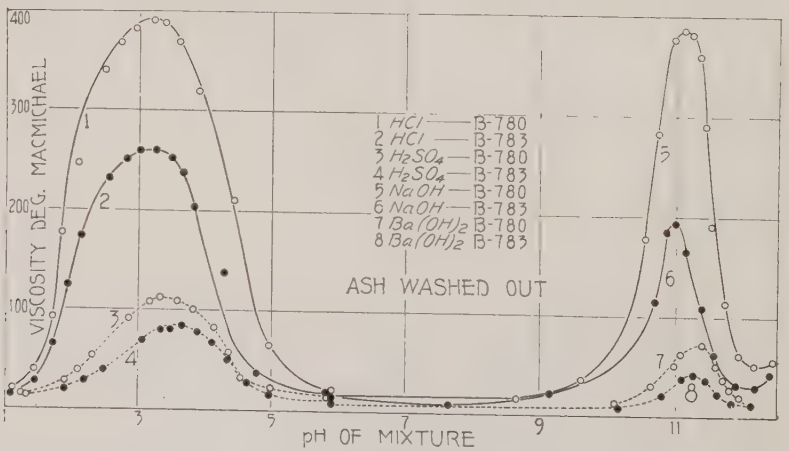

Fig. 9. Relation of Viscosity and Hydrogen-ion Concentration with Extracted Flour

TABLE XI

VISCOSITY, IN DEGREES MACMICHAEL, OF FLOURS B-780 AND B-783 AS INFLUENCED BY THE ADDITION OF VARIOUS AMOUNTS OF NORMAL SODIUM AND BARIUM HYDROXIDE SOLUTIONS, WITH THE pH OF THE RESULTING MIXTURE*

The water soluble substances present in the natural flour were largely removed by digesting 18 grams of flour on the dry basis with 1000 cubic centimeters of distilled water for 45 minutes at 25° C. with occasional shaking. The supernatant liquid was removed by decantation, the residue was treated with 500 cubic centimeters of distilled water, shaken, allowed to stand 15 minutes, and the decanted material remaining was made up to a volume of 100 cubic centimeters with distilled water. The viscosity values have all been corrected for dilution back to the original volume of 100 cubic centimeters. All determinations were carried out at the temperature of 25° C. The wire in this case was standardized with a 60 per cent sucrose solution whose absolute viscosity is 43.86 centipoise. This solution gave a reading of 87.7 degrees MacMichael.

cc. of N. alkali added	Sodium hydroxide				Barium hydroxide			
	B-780		B-783		B-780		B-783	
	Visc.	pH	Visc.	pH	Visc.	pH	Visc.	pH
0.00	15	...	9	...	15	...	9	...
0.20	15	8.66	9	7.64	15	...	9	...
0.40	36	9.62	21	9.16	15	...	9	...
0.80	177	10.55	116	10.68	15	10.09	9	10.14
1.00	282	10.72	184	10.85	...	...	...	...
1.20	339	...	194	11.02	32	10.63	22	10.80
1.40	378	10.94	...	...	...	...	...	...
1.60	387	11.11	164	11.11	50	10.97	39	11.06
1.80	381	11.23	...	...	...	...	...	...
2.00	358	11.34	107	11.41	63	11.09	43	11.26
2.25	290	11.45	...	...	64	...	43	11.31
2.50	190	11.53	63	11.56	68	11.28	...	...
3.00	112	11.75	42	11.72	73	11.39	38	11.45
4.00	62	11.92	33	11.87	51	11.58	22	11.61
5.00	...	...	...	...	38	11.70	...	...
6.00	51	12.16	31	12.17	27	11.78	16	11.83
8.00	...	...	...	...	21	11.94	...	...
10.00	55	12.41	44	12.41	14	12.10	14	12.10

* In case of barium hydroxide it was, of course, necessary to use a more dilute solution. The data have all been corrected back to the basis of a normal solution, however.

MAXIMUM VISCOSITY OBTAINED WITH LACTIC ACID USING A SERIES OF DIFFERENT FLOURS

The determination of viscosity with the salts partially removed probably represents more nearly the actual imbibitional powers of the proteins present in the flour. This is essentially a study of gluten, and hence those variables in its environment (such as electrolytes) which affect gluten properties should be removed, or rendered uniform. The ash content of flours (even of the higher grades) is so variable as to occasion corresponding variations in the imbibitional properties of the gluten, and the soluble ash components should accordingly be removed before gluten comparisons are attempted. It was therefore thought probable that some relation could be found between the viscosity values of flours and their baking strength. Lactic acid is probably the one

best suited for this study because it maintains approximately maximum viscosity over a wide range of acid concentration (see Figures 1 and 2). Mr. Marshall, of the United States Bureau of Markets, kindly supplied a series of flours for this study, together with the baking and analytical data. The high points of the curve reached by the addition of increasing amounts of lactic acid were determined both with and without the removal of the water-soluble extract. These data and the baking and analytical data supplied by Mr. Marshall, are given in Table XII.

It is to be regretted that the viscosity data in some instances may not be the exact values, for there was not enough flour in most cases to run duplicate determinations.

EFFECT OF CONCENTRATION OF FLOUR ON VISCOSITY

In this study 12, 15, 18, 21, and in some instances 24 grams of flour on the dry basis were made up to 100 cc. with distilled water, allowed to stand 1 hour, and the increase in viscosity produced by the addition of various amounts of concentrated syrupy lactic acid was determined. The highest viscosity obtained by the addition of lactic acid is given in Table XIII, together with the actual grams of crude protein and of glutenin contained in the portions of flour used. This table is also made to include the grams of crude protein and glutenin in the portions of flour used in Table XV.

It was found that if the logarithms of the viscosity readings were plotted as ordinates and the logarithms of the flour concentration as abscissa, straight lines were obtained, as shown in Figure 10. Figure 10 also contains the logarithms of the viscosity readings plotted against the logarithms of the crude protein content and the glutenin content of the portions of flour used.

It will be observed from Figure 10 that the lines do not all lie at the same angle with respect to the axis of abscissa.

The equation for the logarithmic curve is

$$\text{log. viscosity} = a + b\ (\text{log. concentration})$$

where a is the logarithm of the viscosity reading when log. of concentration is zero; and b is the tangent of the angle made by the logarithmic curve with the axis of abscissa. The equation may also be expressed in the following form:

$$\text{Viscosity} = a\ (\text{concentration})^b$$

The constants a and b were calculated from the data by the method of least squares. The values of the constant b were also measured from the curve. The results of these calculations are given in Table XIV.

TABLE XII

ANALYSES OF FLOUR SAMPLES REPRESENTING TWO RANGES OF CRUDE PROTEIN CONTENT

The flours are arranged within each group in the order of their loaf volume. The highest viscosity obtained with lactic acid, both with and without the removal of the soluble salts, is given in the last two columns. The milling, baking, and analytical data were supplied by Mr. Marshall.

Lab. No.	U. S. supervisor's grade	Straight flour yield, %	Water absorption, %	Loaf volume cc.	Weight of loaf, gms.	Color of bread, score	Texture of bread, score	Protein (N × 5.7) 13.5 % moisture		Ash in flour %	M.° extract removed*	M.° extract present*
								% in wheat	% in flour			
				11% to 12%	crude	protein in flour						
6905	5 Dark Northern Spring....	67.2	61.5	2400	507	93.0	83.0	15.7	11.4	0.52	515	247
6632	Hard Red Winter.....	74.5	58.8	2270	493	94.5	95.0	12.4	11.3	0.48	338	151
6806	2 Hard White	69.4	60.0	2210	503	90.0	90.0	12.4	12.0	0.48	294	97
6727	5 Dark Northern Spring....	62.7	57.9	2180	498	90.5	91.5	10.8	11.9	0.46	382	122
6918	3 Dark Hard Winter.....	70.0	66.5	2170	522	94.0	94.0	14.7	11.2	0.41	454	269
6584	Hard Winter	69.1	58.5	2160	500	87.5	91.0	12.3	11.2	0.43	257	136
6982	1 Dark Northern Spring....	72.6	62.6	2120	517	88.5	90.0	13.3	12.0	0.46	368	194
7017	1 Dark Northern Spring....	74.1	60.6	2110	509	92.0	90.0	12.9	11.5	0.46	375	187
6989	1 Dark Northern Spring....	73.4	60.6	2100	511	90.5	88.5	12.5	11.3	0.49	356	145
6725	3 Dark Northern Spring....	67.1	56.5	2090	497	87.0	92.0	11.7	11.4	0.42	393	121
6723	2 Dark Northern Spring....	70.9	55.6	1990	494	86.5	89.0	11.8	11.2	0.43	418	119
6857	3 Soft White	71.7	58.5	1980	502	89.0	88.0	13.7	11.7	0.40	312	112
6730	2 Amber Durum	73.7	64.7	1920	517	88.5	92.5	11.5	11.1	0.68	204	47
6769	2 Dark Hard Winter.....	72.4	60.9	1870	512	91.0	87.5	14.9	11.8	0.43	430	145
				8% to 9%	crude	protein in flour						
6960	1 Hard Winter	70.2	62.1	2290	501	93.5	92.8	12.1	8.1	0.46	311	135
6965	5 Hard Winter	71.9	60.9	2210	498	88.5	87.0	12.5	9.0	0.57	334	147
6966	2 Dark Hard Winter.....	71.7	62.4	2170	506	92.0	92.0	12.9	8.8	0.53	391	166
6977	1 Hard Winter	74.3	61.5	2140	506	92.5	91.5	11.7	8.7	0.52	264	155
6649	1 Mixed	71.3	58.8	2040	510	95.0	89.5	10.0	8.9	0.46	263	96
6641	1 Soft White	75.5	61.2	2010	514	95.0	88.5	9.3	8.3	0.51	263	81
6642	1 Hard White	75.5	63.5	1990	513	95.0	86.5	9.9	8.6	0.54	215	66
6643	1 Soft White	76.5	55.9	1980	493	90.0	86.5	9.2	8.0	0.47	157	50
6637	1 Hard Winter	75.4	59.1	1920	507	91.5	88.5	8.9	8.2	0.49	274	89
7056	1 Soft White	70.1	57.1	1920	497	91.5	91.5	10.0	8.9	0.47	170	66
7054	2 Hard White	74.7	65.3	1890	516	90.0	89.0	8.9	8.5	0.68	320	67

* M.° signifies readings in degrees MacMichael.

TABLE XIII

HIGHEST VISCOSITY REACHED BY SUSPENSIONS OF DIFFERENT AMOUNTS OF VARIOUS FLOURS ON THE ADDITION OF CONCENTRATED SYRUPY LACTIC ACID, TOGETHER WITH THE GRAMS OF CRUDE PROTEIN AND GLUTENIN CONTAINED IN THE INDICATED PORTIONS OF FLOUR

The weighed portions of flour on the dry basis were made up to a total volume of 100 cc. with distilled water and digested with occasional shaking for 1 hour before the determinations were made. The determinations were all made at 25° C. A 60 per cent sucrose solution gave a viscosity reading of 29.2° M. with the setting of the wire used.

Flour Lab. No.	6 gms. flour		9 gms. flour		12 gms. flour		
	Protein, grams	Glutenin, grams	Protein, grams	Glutenin, grams	Protein, grams	Glutenin, grams	Viscosity, M°
1001	0.763	0.342	1.144	0.513	1.525	0.684	17
1002	0.874	0.393	1.312	0.590	1.748	0.786	19
1003	0.599	0.251	0.898	0.377	1.198	0.503	6
1004	0.843	0.367	1.265	0.550	1.686	0.733	7
1005	0.738	0.326	1.106	0.489	1.475	0.652	21
1006	0.804	0.329	1.205	0.493	1.607	0.658	30
1007	0.941	0.388	1.411	0.583	1.882	0.776	20
1008	1.027	0.447	1.540	0.671	2.053	0.894	36
1009	0.925	0.414	1.387	0.622	1.849	0.830	32
1010	0.699	0.303	1.049	0.455	1.398	0.606	10
1011	0.720	0.296	1.080	0.444	1.440	0.592	8

Flour Lab. No.	15 gms. flour			18 gms. flour		
	Protein, grams	Glutenin, grams	Viscosity, M°	Protein, grams	Glutenin, grams	Viscosity, M°
1001	1.907	0.855	40	2.288	1.026	79
1002	2.186	0.983	34	2.623	1.179	57
1003	1.497	0.629	12	1.796	0.754	21
1004	2.108	0.917	13	2.529	1.100	24
1005	1.844	0.815	40	2.212	0.977	67
1006	2.009	0.822	67	2.410	0.986	106
1007	2.352	0.971	41	2.822	1.165	74
1008	2.567	1.118	65	3.080	1.341	106
1009	2.312	1.038	72	2.774	1.246	136
1010	1.748	0.758	21	2.097	0.909	37
1011	1.800	0.740	15	2.160	0.887	25

Flour Lab. No.	21 gms. flour			24 gms. flour		
	Protein, grams	Glutenin, grams	Viscosity, M°	Protein, grams	Glutenin, grams	Viscosity, M°
1001	2.669	1.197	135	....	....	..
1002	3.060	1.376	85	....	....	..
1003	2.096	0.880	32	2.395	1.006	49
1004	2.951	1.283	33	3.372	1.466	50
1005	2.581	1.140	104	2.950	1.303	140
1006	2.812	1.151	169	....	....	..
1007	3.293	1.359	109	....	....	..
1008	3.593	1.565	158	....	....	..
1009	3.236	1.453	198	....	....	..
1010	2.447	1.061	53	2.796	1.212	83
1011	2.520	1.035	39	2.880	1.183	55

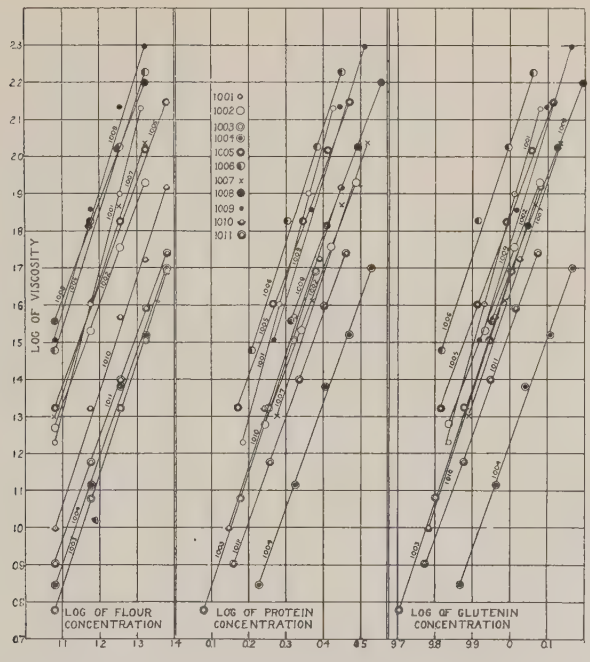

Fig. 10. A. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Flour Concentration of Unextracted Flour
B. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Protein Concentration of Unextracted Flour
C. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Glutenin Concentration of Unextracted Flour

TABLE XIV

CONSTANTS FOR THE LOGARITHMIC EXPRESSION: LOG VISCOSITY $= a + b$ (LOG CONCENTRATION) WITH THE SALTS PRESENT

The calculated values were obtained by the method of least squares, the values for a are given on the basis of flour concentration, crude protein concentration, and glutenin concentration. The measured values for b obtained by the graphical method are also given.

Flour Lab. No.	Measured value of b	Calculated values			
		b	Flour conc. a	Protein conc. a	Glutenin conc. a
1001	3.647	3.705	—2.671	—0.558	—1.848
1002	2.662	2.689	1.799	0.570	1.555
1003	2.980	3.013	2.469	0.547	1.685
1004	2.852	2.829	2.184	0.207	1.232
1005	2.691	2.766	1.654	0.864	1.845
1006	2.955	2.996	1.731	0.883	2.048
1007	3.043	3.052	1.982	0.473	1.646
1008	2.620	2.643	1.295	0.732	1.686
1009	3.341	3.291	2.028	0.645	1.791
1010	2.858	3.005	2.227	0.578	1.669
1011	2.729	2.797	2.114	0.461	1.542

The effect of the removal of the soluble materials on the change in viscosity with concentration was also determined. For this purpose 6, 9, 12, 15, 18, and in some instances 21 grams of flour on the dry basis was digested with one liter of water, with occasional shaking, for 45 minutes, and then allowed to stand until the material had settled, from 5 to 15 minutes. The supernatant liquid was decanted and the residue made up to a volume of 500 cc. with distilled water, shaken, and the material allowed to settle. The supernatant liquid was again decanted and the residue made up to a total volume of 100 cc. and the maximum viscosity attainable with normal lactic acid determined. This substitution of simple decantation for the longer and more tedious procedure of removing the supernatant liquid by means of the centrifuge was adopted only after it was found that the two methods gave results which agreed within experimental error. Duplicate determinations were made throughout. The highest viscosity obtained by the addition of various amounts of normal lactic acid is given in Table XV.

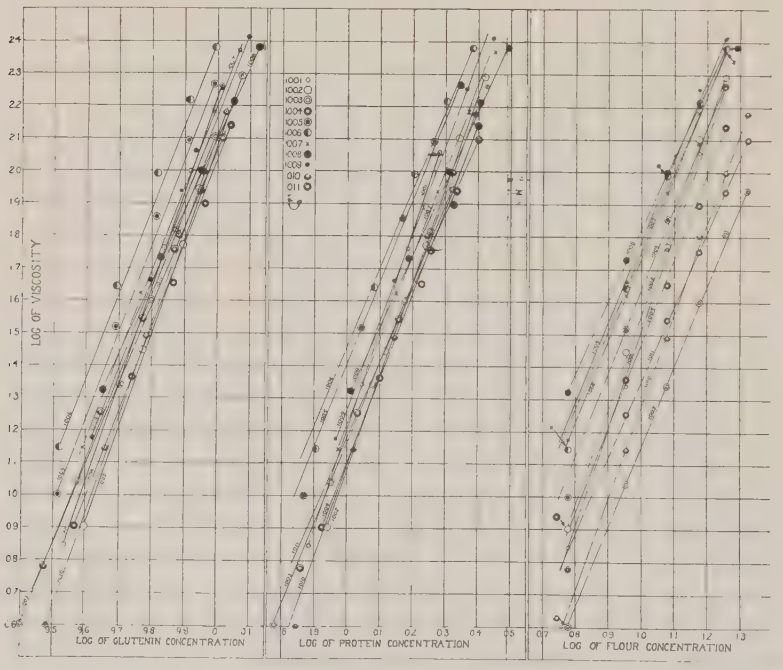

Fig. 11. A. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Glutenin Concentration of Extracted Flour
C. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Protein Concentration of Extracted Flour
B. Logarithms of Highest Viscosity Reading Produced with Lactic Acid Plotted Against Logarithms of Flour Concentration of Extracted Flour

The data in Table XV are expressed in the form of logarithmic curves in Figure 11. In this figure the logarithms of the viscosity as ordinates are plotted against three different abscissa, namely, the logarithms of the flour concentration, of the grams of crude protein present, and of the grams of glutenin present in the portions of flour used. It will be noted that using the glutenin concentration as well as the protein concentration as abscissa produces a closer grouping of the lines indicating the relationship of these components to the imbibition phenomena. It will be noted that the curves with the salts removed lie more nearly at the same angle than is the case with the salts present.

In Table XVI are given the values for the constant b measured from the curves, and the values for b and a calculated by the method of least squares.

TABLE XV

EFFECT OF CONCENTRATION OF FLOUR-IN-WATER SUSPENSIONS ON VISCOSITY

The water soluble substances were removed by digesting the amounts of flour indicated with one liter of distilled water for 45 minutes, decanting the supernatant liquid, shaking with an additional 500 cc. of distilled water, decanting the supernatant liquid, and making the residue up to a total volume of 100 cc. A correction was made for dilution in the viscosity determinations. A 60 per cent sucrose solution having a viscosity of 43.86 centipoise gave a reading of 29.2 degrees MacMichael.*

Flour Lab. No.	6 gms. flour M°	9 gms. flour M°	12 gms. flour M°	15 gms. flour M°	18 gms. flour M°	21 gms. flour M°
1001	7	22	58	115	183	...
1002	8	28	59	127	196	...
1003	4	11	22	40	65	88
1004	8	23	45	79	138	...
1005	10	33	72	124	185	...
1006	14	44	98	165	240	...
1007	14	42	87	153	234	...
1008	21	54	100	163	240	...
1009	15	46	100	180	258	...
1010	4	14	31	64	100	152
1011	6	18	35	57	87	126

* Altho the 60 per cent sucrose solution had the same reading in this table as in Table XIII, a resetting of the wire was made and it is doubtful whether a comparison other than of a relative nature is justified.

Baking data.—The flours used in this work, laboratory Nos. 1001 to 1011, were subjected to a joint investigation by Rumsey (1922), Collatz (1922), and the author. The source, type, and class of the wheat and the grade of the flour are given in Table XVII.

TABLE XVI

CONSTANTS FOR THE LOGARITHMIC EXPRESSION: LOG. VISCOSITY=$a+b$ (LOG. CONCENTRATION) WITH THE SALTS REMOVED

The calculated values were obtained by the method of least squares, the values for a are given on the basis of flour concentration, crude protein concentration, and glutenin concentration. The measured values for b obtained by the graphical method are also given.

Flour Lab. No.	Measured value of b	Calculated values			
		b	Flour conc. a	Protein conc. a	Glutenin conc. a
1001	3.048	3.023	−1.515	+1.193	+2.244
1002	2.954	2.926	+1.365	1.083	2.099
1003	2.492	2.499	1.352	1.157	2.099
1004	2.551	2.552	1.085	1.092	2.011
1005	2.631	2.666	1.048	1.378	2.325
1006	2.654	2.610	0.861	1.418	2.431
1007	2.565	2.568	0.840	1.226	2.213
1008	2.206	2.212	0.396	1.306	2.107
1009	2.678	2.622	0.848	1.281	1.195
1010	2.893	2.909	1.645	1.071	2.127
1011	2.304	2.398	1.062	1.148	2.073

TABLE XVII

HISTORY AND DESCRIPTION OF FLOUR SAMPLES

Flour Lab. No.	Locality where grown	Variety of wheat	Grade of wheat	Grade of flour
1001	Central Kansas Reno County	Turkey Red	Hard Red Winter	Patent
1002	Central Kansas Reno County	Turkey Red	Hard Red Winter	First clear
1003	Washington, Walla Walla	Little Club	White Club	Straight
1004	North Dakota Red River Valley	Marquis and Bluestem	Hard Red Spring	Second clear
1005	North Dakota Red River Valley	Marquis Bluestem	Hard Red Spring	Patent
1006	Canada, Southern Alberta	Marquis	Selected Hard Red Spring	Patent
1007	Canada, Southern Alberta	Marquis	Selected Hard Red Spring	First clear
1008	Canada, Saskat. Valley, Saskatoon	Marquis	Hard Red Spring	Patent
1009	Red River Valley	Marquis mostly	Hard Red Spring	Patent
1010	Utah, irrigated land	Kofod*		Straight
1011	Ohio, Williams County	Like Fultzo-Mediterranean	Soft Red Winter	Long patent

* Identified by J. Allen Clark, of the U. S. Department of Agriculture, as "probably Kofod." Rumsey (1922) and Collatz (1922) refer to this sample as "like Sonora, California wheats." Their identification is undoubtedly incorrect.

The baking data of the series are given in Table XVIII. The data are the result of several bakings in which each flour was baked in such a way as to produce the largest possible volume and at the same time make a loaf of bread that would be desirable commercially. The flours were baked and scored by three bakers over a period of approximately a year, with the same end in view. The results given in Table XVIII are selected with the belief that they represent the most trustworthy data obtained.

PROTEIN FRACTIONS IN THE VARIOUS FLOURS

Because of the slight solubility of gliadin in distilled water it was desirable to ascertain how much protein was removed by the treatment with one liter of water followed by a subsequent treatment with 500 cc. of water. Accordingly the following experiment was carried out. Duplicate portions of 18 grams and 9 grams, on the dry basis, of flour 1009 were treated with one liter of water with occasional shaking, for 45 minutes, the material was allowed to settle for 15 minutes, and the supernatant liquid was then decanted into a 2-liter flask. The residue was treated with 500 cc. of distilled water, shaken thoroly, and allowed to stand 15 minutes, then the supernatant liquid was decanted into the 2-liter volumetric flask, the flask was made up to the mark with water, and the protein content ($N \times 5.70$) of an aliquot was determined by the Kjeldahl method.

The residue from this extraction was treated with 100 cc. of 95 per cent ethyl alcohol, the water already present in the residue being sufficient to dilute the alcohol so that the final concentration was approximately 70 per cent alcohol. This material was then digested for two hours with occasional shaking, at the end of which time it was centrifuged and the supernatant liquid decanted into a 500-cc. volumetric flask. The residue was then treated wih 100 cc. of 70 per cent alcohol and digested, with occasional shaking, for 30 minutes. At the end of this time it was centrifuged and the supernatant liquid decanted into the volumetric flask, the extraction of the residue with 100 cc. of alcohol was repeated and the extract added to the volumetric flask. The flask was then made up to volume and the protein content ($N \times 5.70$) of an aliquot was determined by the Kjeldahl method.

The residue from the two extractions was treated with 50 cc. of 70 per cent alcohol, placed in a mechanical shaker, and shaken for 30 minutes. At the end of this time it was centrifuged and the protein content ($N \times 5.70$) was determined by the Kjeldahl method. The residue was again extracted in a similar manner and the protein in the extract determined.

TABLE XVIII

Comparative Baking Tests of Flours*

Data were obtained from the American Institute of Baking, the final score being a summary of points according to the American Institute of Baking standards (1922).

Flour sample	Per cent absorption	Fermentation period, minutes	Proofing period, minutes	Weights in grams					Volumes cc.	Score
				Dough at mixing	Dough molded	Loaf hot	Loaf 1 hour	Loaf 18 hrs.		
1009	59	255	60	564	518	457	448	435	2160	100
1001	58	231	60	543	539	...	488	460	2010	99
1008	60	203	53	542	533	500	492	464	2000	97
1002	58	225	53	532	527	491	...	462	1880	95
1006	61	195	55	541	536	494	486	471	1735	91
1005	59	187	65	536	531	496	484	464	1820	90
1010	58	165	60	531	527	491	482	468	1760	83
1011	56	229	51	529	524	489	479	459	1720	76
1003	53	186	57	520	...	473	464	438	1650	63
1007	65	165	46	557	552	512	500	478	1460	46
1004	58	192	45	549	...	504	498	473	1415	35

* These flours were subjected to a joint investigation by L. A. Rumsey, F. A. Collatz, and the author. The results of the investigation by Mr. Rumsey (1922) and Mr. Collatz (1922) are presented by them.

The residue remaining after this extraction was treated twice with 50 cc. of 95 per cent ethyl alcohol by shaking in a mechanical shaker for 30 minutes and followed by a treatment with 50 cc. of ethyl ether. The protein content of these combined extracts was determined.

The final residue was allowed to dry in the air and was then treated with enough distilled water to make a total volume of 100 cc., and the effect of normal lactic acid on its viscosity was determined. The highest value obtained with the various amounts of lactic acid is given in Table XIX, together with the data on the protein extracted.

It will be noted that while the viscosity reached is not as high as that reached in the previous determination, i.e., 258° M., (cf. Table XV) the increase is of the same order of magnitude and confirmed earlier indications that the glutenin was mainly responsible for the imbibitional effects produced by the addition of acid.

In order to investigate this point still further, 18 grams of flour 1009 was shaken up with 500 cc. of distilled water, the flour particles allowed to settle for 15 minutes, and the supernatant liquid then decanted into a 2-liter volumetric flask. Extraction with 500 cc. of distilled water was repeated three times, making a total volume of 2 liters in four separate portions used for the extraction. The protein content ($N \times 5.70$) was determined on an aliquot of the extract.

The residue from the above extraction was again extracted with 2 liters of distilled water in the way described and the amount of protein ($N \times 5.70$) removed in this second extraction was determined by the Kjeldahl method.

The residue remaining after this extraction was treated with 100 cc. of 95 per cent ethyl alcohol, shaken in a mechanical shaker for one hour, centrifuged, and the extract decanted into a 500-cc. volumetric flask. The residue was twice extracted with 100 cc. of 70 per cent ethyl alcohol and the protein content determined on an aliquot of the combined extracts.

The same procedure was repeated with a second sample of flour, except that 12 extractions with water were made previous to the alcohol extraction.

The same procedure was repeated with a third sample of flour, except that 16 extractions were made with water previous to the alcoholic extraction. The results are reported in Table XX together with the highest viscosity reached when the final residue, made up to 100 cc. with water, was treated with various amounts of lactic acid.

TABLE XIX

PROTEIN EXTRACTED FROM FLOUR 1009 BY VARIOUS METHODS

(1) One liter of water followed by 500 cubic centimeters of water, (2) residue extracted 3 times with 70 per cent alcohol, (3) sum of (1) and (2), (4) residue extracted with 50 cubic centimeters of 70 per cent alcohol, (5) residue extracted again with 50 cubic centimeters of 70 per cent alcohol, (6) residue extracted twice with 50 cubic centimeters of 95 per cent alcohol followed by 50 cubic centimeters of ether, (7) sum of protein extracted, (8) dry residue was made up with water to 100 cubic centimeters volume and the highest viscosity obtainable with lactic acid was determined. Wire read 29.2° M. with 60 per cent sucrose solution of viscosity 43.86 centipoise.

Weight of flour taken, grams	(1) Water extract per cent	(2) First alcohol extract, per cent	(3) (1) + (2) per cent	(4) Second alcohol extract, per cent	(5) Third alcohol extract, per cent	(6) 95% alcohol ether extract, per cent	(7) Total protein extracted, per cent	(8) Viscosity reading, M°
18	3.93	5.16	9.09	0.19	0.13	0.02	9.43	191
18	4.66	4.33	8.99	0.18	0.14	0.02	9.33	195
9	5.88	3.22	9.10	0.17	0.13	0.02	9.42	...
9	6.04	2.83	8.87	0.17	0.11	0.01	9.16	...
		Average=	9.01			Average=	9.33	

Alcohol soluble protein A. O. A. C. method=9.04.

TABLE XX

PROTEIN EXTRACTED FROM FLOUR 1009 BY SUCCESSIVE TREATMENTS WITH WATER FOLLOWED BY ALCOHOL

Eighteen grams of flour was extracted four times with 500 cubic centimeters of water and the protein (N × 5.70) in the combined extract determined. The residue was then extracted again with water the number of times indicated, and finally three times with 70 per cent alcohol. The protein content of the various extracts was determined, the residue made up to 100 cubic centimeters, and the maximum increase with lactic acid determined. Wire used read 29.2° M. with a 60 per cent sucrose solution whose viscosity is 43.86 centipoise.

First 4 extracts, per cent protein	Second 4 extracts, per cent protein	Third 4 extracts, per cent protein	Fourth 4 extracts, per cent protein	Alcohol extracts, per cent protein	Total per cent protein extracted	Viscosity M°
6.67	1.61	...	...	1.85	10.13	*
6.77	1.56	0.75	...	1.25	10.33	157
6.77	1.43	0.63	0.52	1.12	10.33	149

* Residue lost by accident.

It will be seen from Table XX that the extraction with water apparently removed most of the alcohol soluble protein and that the greatest part is removed in the first four treatments with water. The residue did not exhibit as high a viscosity as the original flour when the soluble electrolytes were removed by the process first used, i.e., 258° M. (cf. Table XV). As a result of the treatment recorded in Table XX approximately one per cent more protein was removed than in the case recorded in Table XIX. This would account for the difference in viscosities recorded in the two tables. It was thought that this decrease in viscosity as shown in Tables XIX and XX as compared with Table XV might be due to the denaturizing effect on the glutenin of either the drying or the treatment with alcohol.

In order to investigate this point, 18 grams of flour 1009 was extracted eight times with 500 cc. portions of distilled water followed by three extractions with 70 per cent alcohol as described above, and the residue was then extracted with four additional portions of 500 cc. of distilled water. The amount of protein removed by this last extraction with water was 0.31 per cent. The highest viscosity reached with lactic acid was 202° M.

The procedure was repeated, except that the flour was first extracted with twelve portions of water instead of eight. The amount of protein extracted in the final treatment with water was 0.35 per cent. The highest viscosity reached was 160° M.

The experiment was repeated, except that the flour was first extracted with sixteen portions of distilled water. The protein content of the last water extract was 0.26 per cent, the highest viscosity reached was 105° M. It is rather difficult to interpret these results as, unfortunately, the amounts of protein extracted by the different treatments were not determined. It does seem to indicate that the

treatment with alcohol markedly affects the imbibitional powers of the remaining protein or that the removal of that last portion of protein extracted with the alcohol decreases the imbibition.

An experiment performed with the expectation that it would throw some light on the subject was carried out as follows: 18 grams of flour 1009 was extracted with eight portions of distilled water. The residue was then made up to a total volume of 100 cc. and the effect of additions of various amounts of lactic acid was studied. The experiment was then repeated with 18-gram portions of flour which had been extracted twelve and sixteen times with water.

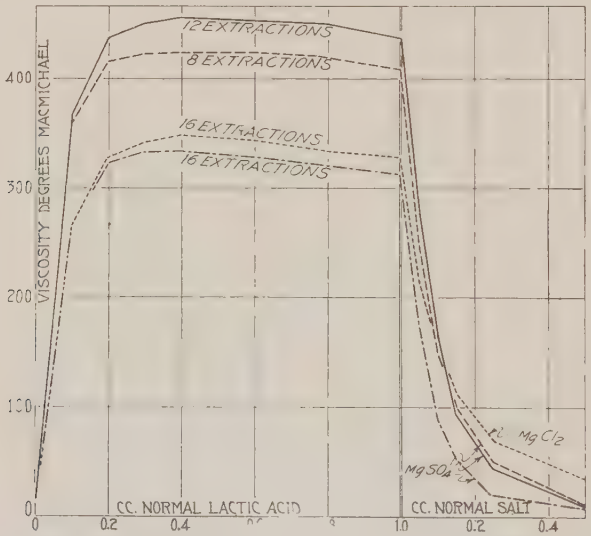

Fig. 12. Effect of Repeated Extraction of the Flour with Water on Viscosity as Influenced by Lactic Acid and Effect of Adding Salts to the System

It will be seen that this experiment is essentially the same as those already noted, except that treatment with alcohol is omitted. The results of the experiment are given in Table XXI and are shown graphically in Figure 12. The results point to some very interesting conclusions. Table XIX shows that eight extractions with water remove 8.28 per cent of protein, twelve extractions remove 9.08 per cent, and sixteen extractions remove 9.35 per cent. Subsequent experiments (Table XXII) indicate that the total sum of the albumen, globulin, and alcohol-soluble protein (gliadin) in sample No. 1009 is 8.49 per cent. It therefore appears that practically all the gliadin has been removed from this flour by repeated extraction with distilled water; nevertheless the maximum viscosity attained, i.e., 456° M., is much higher than any of the previous values, indicating that the

gliadin is not responsible for change in viscosity on the addition of acids but that such changes are probably due almost entirely to the glutenin fraction. The effect of even 0.10 cc. of normal lactic acid is very pronounced, causing an increase in viscosity of more than 90 times the value obtained with distilled water (4° M. to 365° M.). The effect of electrolytes in depressing these high viscosities is equally pronounced, as can be seen from an inspection of Figure 12. We shall have occasion to return to this experiment later when we consider the nature of the mechanism involved in viscosity changes induced by the addition of acids and alkalies.

TABLE XXI

VISCOSITY, IN DEGREES MACMICHAEL, AS AFFECTED BY LACTIC ACID, OBTAINED WITH 18 GRAMS OF FLOUR 1009 BY TREATING THE RESIDUE MADE UP TO 100 CUBIC CENTIMETERS AFTER EXTRACTING 8, 12, AND 16 TIMES WITH 500 CC. PORTIONS OF DISTILLED WATER

After one cc. of lactic acid had been added, the material was treated with normal magnesium sulfate and in one case with magnesium chloride. The wire used read 29.2° M. with a 60 per cent sucrose solution whose viscosity was 43.86 centipoise.

cc. of N. solution added	8 extractions with 500 cc. of water	12 extractions with 500 cc. of water	16 extractions with 500 cc. of water	16 extractions with 500 cc. of water
	Lactic acid			
	M°	M°	M°	M°
0.00	4	4	3	3
0.10	360	365	265	264
0.20	415	436	322	328
0.30	422	452	332	342
0.40	424	456	333	349
0.60	425	453	327	343
0.80	418	449	320	333
1.00	408	439	312	327
	Salt solution added to material containing 1 cc. lactic acid			
	$MgSO_4$	$MgSO_4$	$MgSO_4$	$MgCl_2$
0.05	250	276	177	214
0.10	153	165	90	154
0.15	98	95	53	113
0.25	50	46	22	70
0.50	12	11	7	34

Acting on the hypothesis that the glutenin is the main protein concerned in the imbibition produced by acid and alkali, it appeared very desirable to determine the glutenin content of the flours investigated. As pointed out in the introduction, the accurate determination of the quantity of the various proteins in wheat flour presents unusual difficulties. The data presented in some of the preceding tables confirm this conclusion. In an attempt to determine the proportions of the various proteins in this series of flours, the following determinations

were made. The protein content of the several fractions was calculated by multiplying the nitrogen content as determined by the Kjeldahl method by the factor 5.7.

Total protein.—The total protein was determined on a one-gram sample.

Potassium sulfate–soluble protein (A. O. A. C. method).—For this determination 6 grams of flour was extracted with 100 cc. of 5 per cent potassium sulfate solution by shaking in a mechanical shaking machine for one hour, filtering through a fluted filter, and determining the nitrogen content of a 50-cc. aliquot.

Alcohol-soluble protein (A. O. A. C. method).—In this determination 4 grams of flour was treated with 100 cc. of 70 per cent alcohol by shaking in a mechanical shaker for one hour, filtering the extract clear through a fluted filter, and determining the nitrogen content of a 50-cc. aliquot.

Potassium sulfate-soluble protein, second method.—This procedure was the same as the previous potassium sulfate extraction except that at the end of the shaking period the material was centrifuged and the volume of the supernatant liquid measured. The supernatant extract was then filtered through a fluted filter and the nitrogen in a 50-cc. aliquot determined.

Alcohol-soluble protein in the residue after extraction with potassium sulfate.—Enough alcohol was added to the residue after extraction with 5 per cent potassium sulfate solution to make a total volume of 150 cc. of 70 per cent alcohol, and the mixture was digested by shaking in the mechanical shaker for one hour. At the end of this time the material was allowed to settle and was then filtered clear through a fluted filter, and the nitrogen in a 50-cc. aliquot was determined.

In the case of all these filtrations the first portion of liquid running through the filter was returned to the filter.

All the above determinations were carried out in duplicate, and if the duplicates did not show close agreement the determination was repeated.

It was believed that the glutenin content would be represented most accurately by subtracting from the total protein content of the flour the sum of the amounts of protein extracted by 5 per cent potassium sulfate followed by 70 per cent alcohol. The A. O. A. C. method of determining glutenin in flour is to subtract from the total protein the sum of the potassium sulfate–soluble and alcohol-soluble protein as determined on different portions of flour. As these two solutions have been shown partially to extract the same material, the glutenin value obtained by this method is much too low.

TABLE XXII

PROTEIN FRACTIONS OF VARIOUS FLOURS IN TERMS OF PERCENTAGE OF TOTAL FLOUR ON DRY BASIS. ARRANGED IN ORDER OF THEIR LOAF VOLUME

(1) Lab. No.	(2) Loaf volume, cc.	(3) 5 per cent K_2SO_4 soluble protein, per cent	(4) 5 per cent K_2SO_4 soluble protein centrifuged, filtered, per cent	(5) Alcohol-soluble protein, per cent	(6) Alcohol-soluble after K_2SO_4 extraction, per cent	(7) Total protein content, per cent	(8) Dry hand-washed gluten, per cent	(9) Glutenin (7)– (4)+(6)], per cent
1009	2160	1.59	1.69	9.04	6.80	15.41	15.9	6.92
1001	2010	1.36	1.55	6.81	5.46	12.71	11.7	5.70
1008	2000	1.95	2.11	10.09	7.55	17.11	16.6	7.45
1002	1880	1.74	1.99	7.99	6.03	14.57	14.2	6.55
1005	1820	1.54	1.74	7.41	5.12	12.29	11.5	5.43
1010	1760	1.63	1.76	6.96	4.84	11.65	11.5	5.05
1006	1735	1.54	1.71	7.98	6.20	13.39	12.7	5.48
1011	1720	1.70	1.91	7.33	5.15	12.00	11.7	4.94
1003	1650	1.21	1.37	5.75	4.42	9.98	9.7	4.19
1007	1460	2.20	2.35	8.98	6.86	15.68	15.6	6.47
1004	1415	2.46	2.74	7.55	5.20	14.05	12,9	6.11

The results for the potassium sulfate–soluble protein by the two methods do not agree, altho the only difference in manipulation was filtration of the liquid in one instance, and the use of a centrifuge for a preliminary classification preceding filtration in the other. It is probable that in the case of filtration without centrifuging, the starch and suspended matter have not completely settled and the filters, therefore, clog and ultra-filter out some of the protein in solution. When the centrifuge is used, this suspended material is thrown down, thus preventing the clogging of the pores of the filter paper. There is a marked difference in the rate of filtration in the two instances, filtration being very much more rapid following centrifuging. These experiments indicate the desirability of carefully studying the methods for the determination of the amount of the various proteins in cereal products.

The data obtained are shown in Table XXII. The dry crude gluten, washed from the flour by hand, is included in this table.

EFFECT OF TEMPORARY CHANGES IN HYDROGEN-ION CONCENTRATION OF DOUGH ON COLLOIDAL PROPERTIES OF GLUTEN

The flour used in this experiment was a strong patent having an absorption of 56.2 per cent.[1] The amount of hydrochloric acid required to bring the dough to a pH of 3.0 and of sodium hydroxide to bring a dough to a pH of 11.0 was determined. These two hydrogen-ion concentrations were chosen because they were the points of maximum imbibition with acid and alkali respectively. Doughs were then made with water containing enough acid or alkali to give the one or the other of these hydrogen-ion concentrations, but the amount of water was restricted, so that when the acid or alkali was neutralized with an equivalent solution, the dough would contain the normal amount of water—56.2 per cent. The doughs were kept at these two different hydrogen-ion concentrations for 30 minutes and then the acid or alkali was neutralized, bringing the dough back as nearly as possible to its original hydrogen-ion concentration, and additional water was added to give the necessary dough consistency. The amount of sodium chloride formed by this neutralization was subtracted from the amount of salt added in the baking formula. The other ingredients of the dough were not added until after the acid or alkali had been neutralized. The baking formula and procedure used was that suggested by Bailey (1916). Two duplicate doughs were prepared in each case and a predetermined amount of takadiastase was added to one in order that the diastatic activity might not be a limiting factor. In another set of

[1] The absorption of a flour is usually considered 56.2 per cent when 56.2 grams of water are added to 100 grams of air-dry flour to make a dough of the required consistency.

experiments one half the dough was brought to a pH of 3.0, the acid neutralized, and then the other half of the flour added.

The actual absorption of a dough at a pH of 3.0 was determined and was found to be 61.3 per cent. While this is an increase of about 5 per cent over the control, nevertheless the increase is small compared with what might be expected from the viscosity curves. The reason for this small difference may be due to two causes: The inhibiting effect of the sodium chloride added in the baking formula, and the large amounts of imbibitional water which may not be held firmly enough to make a dough of the proper consistency. The results of the experiments are given in Table XXIII.

TABLE XXIII

EFFECT ON LOAF VOLUME OF TEMPORARILY CHANGING THE HYDROGEN-ION CONCENTRATION OF THE DOUGH

For each loaf 300 grams of flour was taken.*

Treatment of dough	Loaf volume, cc.	Absorption, per cent
Control	1600	56.2
To pH 3 and back	830	59.1
To pH 3 and back+diastase	1050	61.8
To pH 11 and back	1130	58.9
To pH 11 and back+diastase	1085	56.2†
Absorption determined at pH 3	....	61.3
New Baking		
Control	1425	56.8
Control+diastase	1390	57.2
Half of dough to pH 3 and back	1325	63.4
Half of dough to pH 3 and back+diastase	1345	63.9

* Mr. Arnold Johnson kindly carried out the bakings reported in this table.
† Amount of water restricted.

It will be noted that all the treatments decreased the loaf volume. The decrease is not so apparent in the second set of bakings. In this baking the control did not behave normally. This the baker attributed to the poor quality of the yeast used. The effect of the treatments was shown very markedly in the handling of the doughs. They were extremely short and broke like putty when pulled, while the baked bread had a very coarse texture. The loaves in which the hydrogen-ion concentration had been changed had practically white crusts, exhibiting no browning in the oven, altho the control loaves browned to an unusual extent. The addition of diastase did not alter this condition.

We have found in other experiments that the viscosity of a flour-in-water suspension at a pH of 11.0 decreases fairly rapidly with time of standing. Keeping a dough at a pH of 11.0 for 30 minutes may permit the alkali to exert its peptizing action on the gluten and thus destroy the colloidal properties which give the flour its desirable baking quali-

ties. A flour-in-water suspension kept at a pH of 3.0 for one hour shows no appreciable change in viscosity due to standing, nevertheless a dough held at this hydrogen-ion concentration for 30 minutes lost its baking strength. At this hydrogen-ion concentration the gluten may have undergone changes of a nature similar to solution which would result in a decided change in its colloidal properties.

EFFECT OF TREATMENT WITH ALCOHOL ON COLLOIDAL CONDITION OF THE GLUTEN AS REFLECTED IN LOAF VOLUME

Gliadin has been universally regarded as an important factor in flour strength because whenever the gliadin has been removed from flour by extraction with alcohol and the resulting residue dried and remilled, the flour so obtained was found to have lost the capacity "for producing a bold, large-volumed, well-risen loaf."

Snyder (1897), Voorhees (1900), and Olson (1917) all demonstrated the loss in baking strength due to the removal of gliadin from the flour, but apparently no one of these observers tried the experiment of treating flour with alcohol and then drying, remilling, and baking the flour without removing the alcohol-soluble protein. Kosutány (1903) was able to obtain only 5.5 per cent of wet gluten of very poor quality from flour which had been simply treated with alcohol and then redried. The effect of alcohol on the colloidal properties of the glutenin as shown by the viscosity studies (cf. Tables XX and XXI) suggested the following experiment:

Doughs were prepared using 70 per cent and, in two instances, 95 per cent ethyl alcohol, 60 cc. of alcohol being added per 100 grams of flour. The doughs so prepared were crumbly and not coherent. As soon as the doughs were made, they were crumbled and placed on large glass plates. A current of air was blown over the material with an electric fan and it was continuously crumbled and stirred. The flour was to all appearances dry within an hour, the drying taking place at a temperature not exceeding 25° C. The material so obtained was remilled in an experimental flour mill and baked. Check samples of normal untreated flour were baked at the same time. The experiment was carried out with the patent flour used in the experiments reported in Table XXIII and with samples of both durum and rye flours. The rye flour contains no protein which responds in any appreciable degree to the imbibitional effect of acids, and it was chosen because it was believed that if flour strength was associated with the colloidal condition of the glutenin, treatment with alcohol would not affect the baking

qualities of the rye flour to an appreciable extent. The results obtained are given in Table XXIV. Photographs of the resulting loaves are shown in Plates I and II.

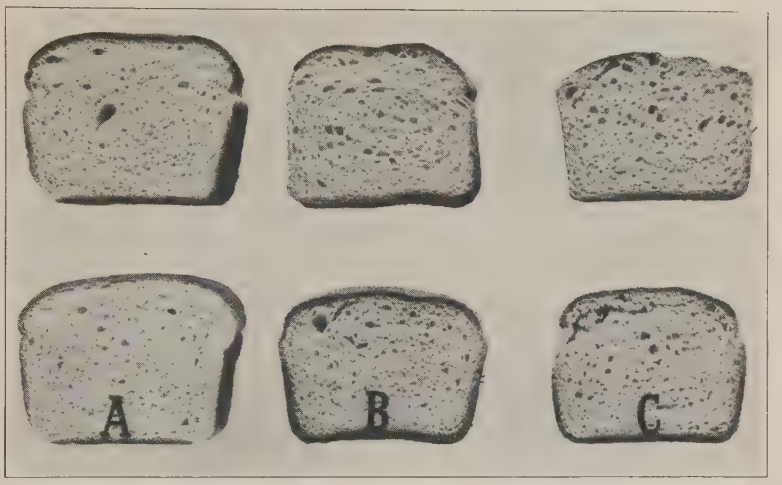

Plate 1. Effect of Alcohol Treatment on Baking Qualities of Wheat Flour (See Table XXIV.)
A. Check, normal flour.
B. Flour doughed 95% alcohol, dried, remilled, and baked.
C. Flour doughed 70% alcohol, dried, remilled, baked.

TABLE XXIV

EFFECT ON BAKING STRENGTH OF FLOUR OF PREPARING DOUGHS WITH ALCOHOL, IMMEDIATELY DRYING AT 25°C., REMILLING, AND BAKING

The controls in each case were normal, untreated flours*

Kind of flour	Conc. of alcohol, per cent	Diastase added	Loaf volume, cc.	Absorption, per cent
White flour control	..	No	1425	56.8
White flour control	..	Yes	1390	57.2
White flour	95	No	1170	59.2
White flour	95	Yes	1230	60.3
White flour	70	No	1235	60.6
White flour	70	Yes	1190	58.8
Durum control	..	No	1605	58.6
Durum control	..	Yes	1515	58.1
Durum	70	No	1280	66.9
Durum	70	Yes	1205	66.9
Rye control	..	No	1145	52.0
Rye control	..	Yes	1160	52.0
Rye	70	No	1310	55.4
Rye	70	Yes	1300	55.4

* Mr. Arnold Johnson kindly carried out the bakings reported in this table.

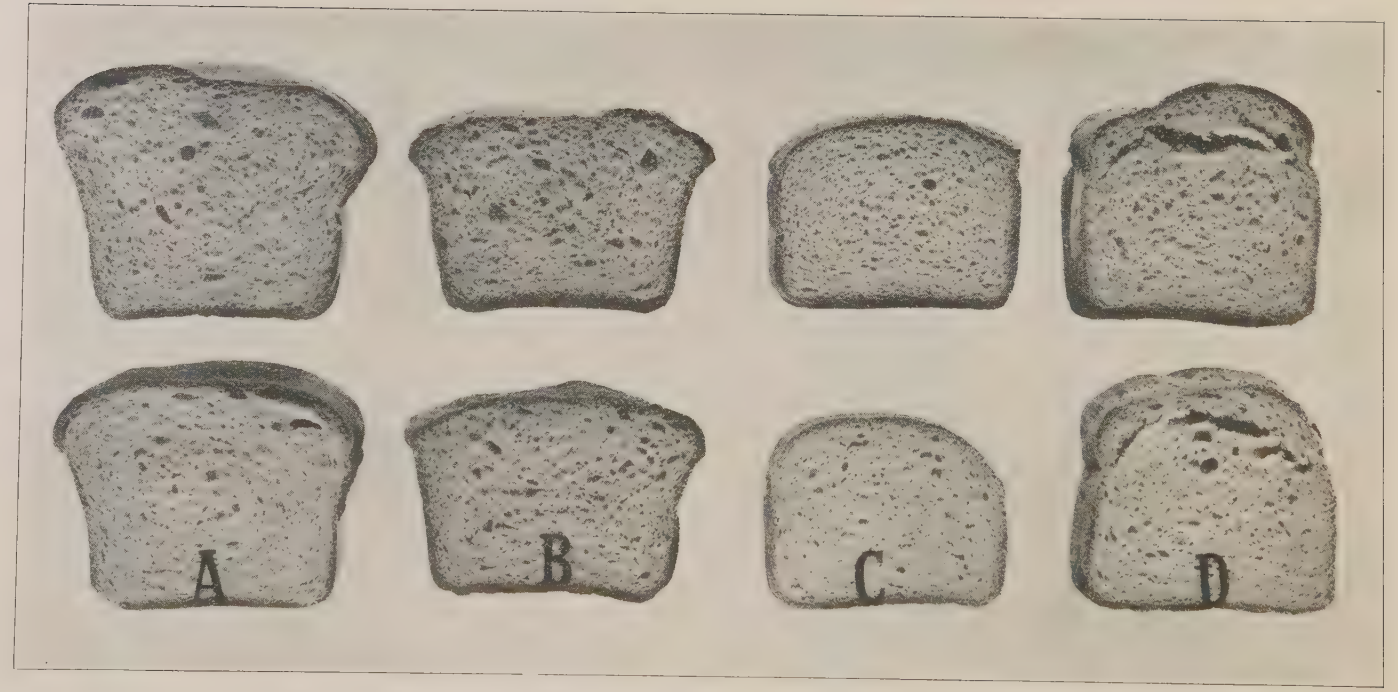

Plate II. Effect of Alcohol Treatment on Baking Qualities of Durum Wheat Flour as Contrasted with Rye Flour (See Table XXIV for data.)

A. Check loaf, durum flour.
B. Durum doughed 70% alcohol, dried, remilled, and baked.
C. Rye check.
D. Rye doughed 70% alcohol, dried, remilled, and baked.

Fermentation progressed at an apparently normal rate in the doughs which had been previously treated with alcohol, altho after the first punch they did not rise as high as the controls. The alcohol-treated patent flour and durum flour doughs were hard to handle; they were short and puttylike and broke in the kneading process. Of the rye flour doughs, no difference could be detected between the control doughs and those made from alcohol-treated flour.

Small open craters were formed toward the end of the fermentation of the alcohol-treated doughs, owing to the loss of carbon dioxide through the surface of the fermenting mass. These craters did not close after forming, and the surface of the doughs looked as if perforated with a blunt instrument.

These experiments are taken as rather definite proof that the colloidal properties of the gluten are extremely important in the baking quality of a flour. In these experiments no material was removed from the flour, the flour was left in its original condition as nearly as possible with the exception of changes in its colloidal properties. The amount and relative proportions of the various components are apparently not disturbed. The effect of alcohol was not due to solution of the gliadin, as is shown by the fact that the same deleterious results were produced by 95 per cent alcohol, in which concentration of alcohol gliadin is insoluble. The results obtained in the viscosity experiments indicate that the effect of alcohol may be attributed to change in the colloidal condition of the glutenin.

DISCUSSION

METHODS AND GENERAL OBSERVATIONS

The method adopted in this investigation for the study of the imbibitional power of the proteins in wheat flour was to measure the viscosity of flour-in-water suspensions, and the changes in viscosity produced by various treatments.

At the beginning of the investigation it was found that the viscosity of such suspensions was very sensitive to mechanical treatment, the mere repeating of the viscosity determination with the same sample time after time causing a marked decrease in its viscosity. While this behavior was in agreement with the findings of numerous other investigators, it rendered the investigation more difficult and probably influenced to some extent the accuracy of the results. The viscosity of the suspensions was found to change with time of standing, this being especially noticeable in cases in which the suspension had been treated with acids or alkalies. In an effort to minimize the effect of these factors, the time and mechanical treatment procedures were

standardized as much as possible. The Ostwald viscosimeter did not lend itself readily to such standardization and was therefore abandoned. It would be interesting, however, to study the effect of various pressures on the viscosity of flour-in-water mixtures as shown by a capillary viscosimeter, as the rate of shear was found to have a marked effect on the results obtained. A study of this factor might throw some light on the problem.

The ease of manipulation and the speed with which determinations could be carried out, together with the comparative accuracy with which duplicate determinations could be made, as shown in Table IV, led to the use of a MacMichael viscosimeter for the main part of this work. Because of mechanical construction of the viscosimeter and the effect of different rates of shear on the viscosity readings, the results obtained are only comparative. As the instrument was so constructed that the different parts of the liquid were subjected to different rates of shear, no study of the effect of rate of shear on viscosity of the flour-in-water suspensions would be valid. The results are all expressed in terms of degrees MacMichael because the attempt at expressing them in terms of absolute viscosity would only be misleading, and for that reason no attempt was made to standardize the wire used except to give an approximate idea of the magnitude of the readings in absolute units.

Some might question the value of such extended determinations on the same flours because flours are known to change markedly in baking strength with age. The investigations on these flours were not begun until about six months after the flours had been milled. They were baked at intervals extending over a year. The flour was stored in sealed containers in a cold storage plant at a temperature of about 3° C. The imbibitional powers of the hand-washed crude gluten obtained from flours B-780 and B-783, as measured by increase in weight of discs in the presence of various concentrations of lactic acid, did not change after being kept in storage for two years. The determination was carried out at the beginning and the end of the work with flours B-780 and B-783. For these reasons it is believed that no significant error due to changes in the flour during the progress of these experiments was introduced. It was believed that the investigation would yield more profitable results if a few flours were studied from as many angles as possible rather than fresh flours for each point investigated.

VISCOSITY OF ORIGINAL FLOUR-IN-WATER SUSPENSIONS AS AFFECTED BY VARIOUS AMOUNTS OF DIFFERENT ACIDS

In this investigation the viscosity as affected by a series of normal acids was studied to see if the various acids behaved alike with the different flours. For this study flours B-780 and B-783 were used, the same that were used in the earlier study by Sharp and Gortner (1922).

Table II shows that the increase in viscosity of flour B-780 begins with smaller additions of acid than with B-783. This same effect was observed with all the acids studied in Table III but it was considered sufficient to give only a few examples. As a partial explanation of this difference between the two flours, it should be noted that flour B-780 had an ash content of 0.44 per cent and flour B-783 an ash content of 0.53 per cent. In view of the work of Bailey and Peterson (1921) it would be expected that the addition of the same amount of acid to the two flours would cause a greater change in hydrogen-ion concentration in flour B-780 than in flour B-783. The results presented in Table III show that this is indeed the case.

The results obtained with the various acids are expressed graphically, using in the first instance the cubic centimeters of normal acid added as abscissa (see Figs. 1 and 2) and in the second instance the pH of the various acid solutions as abscissa (see Figs. 3 and 4). The viscosity in degrees MacMichael is used as ordinates in each case. The results are plotted on the different bases in order to show how easy it is to overlook a really fundamental similarity in the behavior of the various acids.

Figures 3 and 4 show clearly that the greatest imbibitional effect which a given acid will produce occurs at practically the same hydrogen-ion concentration regardless of the nature of the acid. This hydrogen-ion concentration appears to be approximately at pH 3.0 for the flour proteins and to be the same within experimental error for the two flours.

That the two flours seem to respond differently to different rates of shear, is shown by a comparison of the values obtained with lactic acid as shown in Tables III and V when different wires were used in the viscosimeter.

If the points of maximum viscosity produced by the various acids with the two flours are compared, it will be noticed that the order of the acids is different in the two flours. Sulfuric acid produces only slight imbibition in the case of flour B-780 and no imbibition in the case of flour B-783. Trichlor acetic acid produces appreciable imbibition with flour B-780 and practically no imbibition with flour B-783.

Meta-phosphoric acid produces no imbibition with either flour. This failure to cause the imbibition of the proteins of the flour is undoubtedly connected with the behavior of these acids as protein precipitants. The experiments with flour proteins would indicate that meta-phosphoric acid would be the most efficient protein precipitant of the acids studied.

EFFECT OF ADDED SALTS ON IMBIBITION PRODUCED BY LACTIC ACID

In these experiments the water with which the flour was treated was made 0.01 normal with respect to several salts and the effect of lactic acid on imbibition was measured by the viscosity method. In the case of dibasic potassium phosphate the concentration used was 0.005 molar. The results are given in Tables VI and VII and are expressed graphically in Figure 5. It was found that magnesium, potassium, and sodium chlorides, and dibasic potassium phosphate all produced about the same decrease in viscosity, both flours behaving similarly in this respect. Magnesium sulfate, potassium sulfate, and calcium chloride were found to decrease the viscosity still more, all three salts decreasing it to about the same extent. The two flours behaved alike in this regard. In the case of the last three salts the beginning of the increase in viscosity is delayed. This is also true with the dibasic potassium phosphate. The effects of these salts fall in very sharply defined groups. The groups are not determined by the valency of the ions, for magnesium chloride falls in the upper group while calcium chloride falls in the lower one. Dibasic potassium phosphate falls in the upper group and magnesium sulfate and potassium sulfate fall in the lower group in spite of the fact that dibasic potassium phosphate is present in slightly greater amount. Potassium and sodium chloride both fall in the same group, the results agreeing with each other within experimental error.

EFFECT OF ELECTROLYTES PRESENT IN THE ORIGINAL FLOUR ON VISCOSITY

It is known that the water extract from wheat flour contains a certain amount of dissolved electrolytes, and it would be expected that they would exert some effect in decreasing the viscosity of the flour-in-water mixture. It has been shown by Bailey and Collatz (1921) that the soluble electrolyte content of a water extract of wheat flour, as measured by conductivity, was related to the ash content of the flour, so the viscosity of a low-grade flour should be depressed more than that of a high-grade flour, owing to the difference in soluble

electrolytes contributed to the extract by the flour itself. Thus the true imbibitional strength of the proteins present would be somewhat masked by the ash content, and flours of different grades contributing different amounts of salts should not be expected to respond alike.

In order to investigate this point, two flours of widely different ash content were chosen. It was found that extracting 18 grams of the flour with one liter of water and centrifuging and discarding the supernatant liquid and then making the residue up to a total volume of 100 cc., increased the viscosity of a flour with a high ash content from 75 to 329 degrees MacMichael, while a flour with a relatively lower ash content increased from 393 to 502 degrees. This confirmed the supposition that the difference in soluble salts of the different flours was changing the order of their relative viscosities. A second extraction with one liter of water was not found to affect the viscosity appreciably. This treatment with one liter of water was found to reduce the salt content as shown by conductivity determinations, to at least one-fifteenth its original value. The subsequent extraction with one liter of water was found to reduce the content slightly further. It was thought, therefore, that this treatment lowered the electrolyte content to a level where it would not affect the results.

EFFECT OF VARIOUS ACIDS ON VISCOSITY OF EXTRACTED FLOURS

In view of the demonstrated effect of the soluble salts, the viscosity determinations were repeated with the various acids on the same flours, B-780 and B-783, after they had been extracted with one liter of water. The results are given in Table IX and Figures 6 and 7. The effect of removing the soluble salts seemed to be to increase the viscosity of B-783 more than that of B-780 and this is just what was expected from the ash content. The acids seemed to behave more nearly alike with the two different flours after the removal of the soluble materials. In order to bring out more clearly the effect of removing the soluble materials, the acids are grouped in Table XXV in order of their power to increase the viscosity of the flour suspension. The table gives the order with flours B-780 and B-783 both with and without the soluble materials. In order to show how the effects of the different acids on the two flours become more nearly alike after the removal of the extract, the maximum points obtained with the various acids are grouped together in Table XXVI, which also gives the ratios of the maximum viscosities as a means of comparison of the two flours. It is seen that in the extracted flours the ratio is more or less constant, while in the unextracted flour it varies considerably. The optimum pH for imbibition in the extracted flours seemed to be slightly on the

TABLE XXV

ION SERIES AT MAXIMUM VISCOSITY FOR FLOURS B-780 AND B-783, AS SHOWN BY VISCOSITY DETERMINATIONS WITH VARIOUS ACIDS, WITH AND WITHOUT REMOVAL OF WATER-SOLUBLE EXTRACT

Columns begin with the acid showing greatest increase in viscosity. Acids producing approximately the same increase are placed in groups.

Soluble extract present		Soluble extract removed	
B-780	B-783	B-780	B-783
Oxalic	H_3PO_4	H_3PO_4	H_3PO_4
Tartaric			
H_3PO_4	H_3PO_3	HCl	HCl
H_3PO_3	Lactic	HBr	H_3PO_3
		HNO_3	
$CH_2ClCOOH$	HCl		HNO_3
Lactic		$CH_2ClCOOH$	Lactic
	$CH_2ClCOOH$	H_3PO_3	$CH_2ClCOOH$
HCl		CH_3COOH	
	Tartaric		Citric
Citric	Oxalic	Citric	Oxalic
		Lactic	Tartaric
CH_3COOH	CH_3COOH	$CHCl_2COOH$	
	Citric		CH_3COOH
HNO_3		Oxalic	
	HNO_3	Tartaric	H_2SO_4
CCl_3COOH			
	CCl_3COOH	CCl_3COOH	
H_2SO_4			
	H_2SO_4	H_2SO_4	
HPO_3			
	HPO_3	HPO_3	

TABLE XXVI

MAXIMUM VISCOSITY, IN DEGREES MACMICHAEL, PRODUCED WITH VARIOUS ACIDS AND ALKALIES USING FLOURS B-780 AND B-783 BOTH WITH AND WITHOUT REMOVAL OF SOLUBLE EXTRACT

The ratio of the maximum viscosity produced with the same acid on the two different flours is also shown.

Acid	Viscosity salts present			Viscosity salts removed		
	B-780 M°	B-783 M°	B-780 / B-783 ratio	B-780 M°	B-783 M°	B-780 / B-783 ratio
Hydrochloric	273	128	2.14	389	258	1.51
Lactic	280	140	2.00	322	234	1.38
Tartaric	332	118	2.81	305	211	1.45
Oxalic	337	113	2.98	310	211	1.47
Sulfuric	98	33	2.97	111	81	1.37
Acetic	216	105	2.06	333	203	1.64
Nitric	195	66	2.96	373	234	1.59
Citric	247	103	2.40	322	213	1.51
H_3PO_4	332	163	2.04	401	267	1.54
H_3PO_3	332	160	2.08	339	240	1.41
$CH_2ClCOOH$	311	130	2.39	340	230	1.48
CCl_3COOH	147	41	3.58	...	...	...
NaOH	625	342	1.83	387	194	1.99
$Ba(OH)_2$	185	140	1.32	73	43	1.70

alkaline side of pH 3.0. While in many ways the behavior of the different acids appears to be similar, the curves show differences which are still much greater than the experimental errors of the method. It is noted that the freshly prepared meta-phosphoric acid produces no imbibition. This indicates its efficiency as a protein precipitant. The change of meta-phosphoric acid to ortho-phosphoric acid is shown by the effect produced by the acid solution that stood for several months.

EFFECT OF SODIUM AND BARIUM HYDROXIDE ON VISCOSITY OF FLOUR SUSPENSIONS

Barium hydroxide has relatively less effect on the viscosity of the flour suspensions than has sodium hydroxide. This agrees with the findings of Loeb (1921a) and Zoller (1921) for other proteins. Lüers (1919) found that barium and sodium hydroxide increased the viscosity of gliadin solution to practically the same extent. The salts present in the flour repress imbibition in alkalies only to a slight extent, but they exert a marked depressing effect on imbibition in acids, as shown in Figures 8 and 9. The point of maximum viscosity is reached with the two flours at a pH of approximately 11.0, using sodium hydroxide, and at a pH a little higher using barium hydroxide. It was noticed that if the flour in the alkali was kept at its highest point of imbibition for some time, the viscosity decreased with time without the addition of further amounts of alkali. The results obtained with hydrochloric and sulfuric acids are plotted in the same figures to show the effect of a wide range of pH.

The effect of lactic acid on the viscosity of a series of flours was studied in order to see whether or not the viscosity values could be correlated with the baking data. The results showing the maximum viscosity with the extract both present and removed are given in Table XII. Several correlations were made, indicating what appeared to be a relation between the loaf volume, the protein content, and the viscosity values with the extract removed. There were several exceptions, however, indicating that some other factor which had not been taken into account was affecting the results.

EFFECT OF CONCENTRATION ON VISCOSITY

This point was investigated to see whether or not the order of the relative viscosities of the various flours would remain the same if the viscosity effects were measured at different concentrations. The results (Tables XIII and XV) show that concentration does affect the results. It is shown that the effect of concentration on viscosity can be expressed by the equation

$$\log. \text{viscosity} = a + b \log. \text{concentration}$$

It was found that the constant *b*, which is the tangent of the angle made by the logarithmic curve with the axis of abscissa, varied with the different flours. Apparently the value of the constant *b* expressed more nearly the imbibitional strength of the various flours. The values for the constant *a* were found to vary markedly when the concentration was calculated on the basis of grams of flour per 100 cc. If the concentration of the protein content was used the values for *a* more nearly approach each other, while if the concentration was expressed as glutenin the values for *a* show still greater agreement. The values obtained for these constants are given in Tables XIV and XVI. The values for the constants of the curve were determined by the method of least squares. The value of *b* was also obtained by the graphical method.

PROTEIN FRACTIONS IN VARIOUS FLOURS

Gliadin is reputed to have but a slight solubility in distilled water. However, the amount of gliadin removed by water during the extraction of the electrolytes was determined and found to be very appreciable. The results, shown in Table XIX, indicate that it is the alcohol-soluble protein which is removed, for the total amount of protein removed by the water and the subsequent alcohol treatment is only slightly greater than the amount of protein removed by direct treatment with alcohol, i.e., 9.33 and 9.04 per cent, respectively. That it is the alcohol-soluble protein which is removed is indicated by the difference in amounts of protein removed from the 18- and 9-gram samples. More protein was removed by the water treatment and less with the alcohol treatment from the 9-gram sample than from the 18-gram sample.

The results given in Table XX seem to indicate that most of the alcohol-soluble protein can be removed by extraction with water, and that the greatest amount is removed in the first four extractions. In this case the treatment with water and alcohol removed more than 10 per cent of protein, probably including some glutenin. This is highly probable as the material was extracted with water by decantation and some of the glutenin particles might easily have remained in suspension and have been carried over with the decanted liquid, as it was not filtered.

Other observations during the progress of the work indicated that the glutenin is mainly responsible for the imbibitional powers of the proteins of the flour and gluten. This supposition was confirmed by the viscosity determination on the flour from which the gliadin had

been extracted. This material always showed a viscosity of the same order of magnitude as the original flour. The viscosity readings were always considerably lower in the flour from which the gliadin had been extracted by treatment with water followed by alcohol than in material that had not been treated with alcohol. This last treatment with alcohol removed only the small part of the alcohol-soluble protein which had not been removed by water. Thus alcohol shows a marked effect on the viscosity, either by the removal of this last small amount of alcohol-soluble protein or, which seems more probable, by the denaturization of the glutenin, it being well known that alcohol coagulates certain proteins. This latter explanation would be more in agreement with the findings of Sharp and Gortner (1922), who dried crude gluten at a temperature below 50° C. and found that the colloidal properties of the material were markedly altered.

The effect of lactic acid on the viscosity of flour-water suspensions which had been extracted several times with 500 cc. of distilled water was enormous (Table XXI and Fig. 12). The addition of only 0.10 cc. of normal lactic acid was sufficient to increase the viscosity over ninety times its original value. This material, which had reached its maximum point of imbibition by treatment with lactic acid, was very sensitive to the addition of salts, the value decreasing nearly two-thirds on the addition of 0.10 cc. of magnesium sulfate. Magnesium chloride was not so effective in this respect. Another interesting point is that the viscosity obtained was much greater than when the extraction was made with only one liter of water, which gave a value of 258° M., thus indicating that the effect of the electrolytes of the flour had probably not been entirely eliminated by a single extraction with one liter of water.

Because these experiments seem to show that the glutenin is mainly responsible for the imbibitional powers of the flour, the attempt was made to determine the relative amounts of the various proteins in the flours investigated. It was found that centrifuging the 5 per cent potassium sulfate-soluble extract before filtration apparently increased the amount of protein removed in this fraction. It was found that the direct treatment with 70 per cent alcohol removed about the same amount of protein as extracting first with 5 per cent potassium sulfate and then with 70 per cent alcohol. It was believed that the glutenin content would be indicated more accurately by subtracting from the total crude protein the sum of the protein extracted with potassium sulfate and that extracted subsequently with 70 per cent alcohol.

TABLE XXVII

RELATION BETWEEN LOAF VOLUME, GLUTENIN CONTENT, AND THE IMBIBITIONAL STRENGTH OF THE GLUTENIN AS REPRESENTED BY THE CONSTANT *b* OF THE LOGARITHMIC EQUATION

Lab. No.	Loaf volume	Glutenin content	Electrolytes present		Electrolytes removed		Class of wheat	Flour grade
			Constant *b*	$\frac{(\text{Glutenin}) \times (b)}{(\text{Loaf volume})} = K$	Constant *b*	$\frac{(\text{Glutenin}) \times (b)}{(\text{Loaf volume})} = K$		
1009	2160	6.92	3.291	0.01054	2.622	0.00840	Hard Red Spring	Patent
1001*	2010	5.70	3.705	0.01051	3.023	0.00857	Hard Red Winter	Patent
1008	2000	7.45	2.643	0.00985	2.212	0.00824	Hard Red Spring	Patent
1005†	1820	5.45	2.766	0.00828	2.666	0.00795	Hard Red Spring	Patent
1010	1760	5.05	3.015	0.00862	2.909	0.00834	Kofod	Straight
1006‡	1735	5.48	2.996	0.00946	2.610	0.00823	Hard Red Spring	Patent
1011	1720	4.93	2.797	0.00801	2.398	0.00687	Soft Red Winter	Patent
1003	1650	4.19	3.013	0.00765	2.499	0.00635	White Club	Straight
1002*	1880	6.55	2.689	0.00936	2.926	0.01060	Hard Red Winter	First clear
1007‡	1460	6.47	3.052	0.01352	2.568	0.01137	Hard Red Spring	First clear
1004†	1415	6.11	2.829	0.01221	2.552	0.01071	Hard Red Spring	Second clear

* 1001 is a patent and 1002 a clear from the same wheat.
† 1005 is a patent and 1004 a clear from the same wheat.
‡ 1006 is a patent and 1007 a clear from the same wheat.

RELATION OF VISCOSITY, GLUTENIN CONTENT, AND LOAF VOLUME

It has been shown in Figures 10 and 11 that the logarithmic curves do not all lie at the same angle with respect to the axis of abscissa, that is, the viscosity increases more rapidly with concentration in some cases than in others. It is probable that the tangent of the angle of this curve made with the abscissa represents more accurately than any other value thus far obtained, the imbibitional strength of the colloidal protein of the wheat. If the tangent of this angle is small, the colloidal properties will be less marked; on the other hand, if the tangent of the angle has a large value, the colloidal properties will be very pronounced. The constant b in the equation

$$\text{log. viscosity} = a + b \text{ (log. concentration)}$$

represents the tangent of this angle.

Bearing in mind that the tangent b of the angle thus obtained might be considered as the value indicating most accurately the colloidal strength of the flour, and that the imbibition of flour as affected by acids is due mainly to the glutenin, it should be expected that a relationship might exist between these two factors. Thus the colloidal strength of the flour would be the product of the quality factor as indicated by the constant b in the logarithmic equation, and the quantity factor as indicated by the quantity of glutenin present in the flour. In order to see if this product was actually related to the baking strength of the flour as indicated by loaf volume, the former was divided by the latter. The results of this calculation are given in Table XXVII.

Suspensions of various concentrations of flour untreated with acids would doubtless follow the logarithmic relationship and would probably give an angle somewhat similar to the ones obtained in Figures 10 and 11. Unfortunately, the supply of flours used became exhausted, so it was impossible to compare the curves produced with the untreated flours with those given in Figures 10 and 11. The attempt was made to introduce the relationship between the initial viscosity reading of the flour-in-water suspension and the maximum reading after treatment with lactic acid, but the initial reading was so low that it could be determined only approximately with the viscosimeter set for the higher readings, the experimental error being in some cases about 50 per cent. It would seem desirable to investigate this point under conditions in which the lower readings could be determined with sufficient accuracy to subject this relationship to a more exact quantitative study. Likewise the subtraction of the initial value from the maximum value was tried but in some cases this produced relatively large values for the constant b, because some of the flours with low baking strength

yielded simple flour-in-water suspensions which gave relatively high readings; and altho these suspensions increased only slightly in viscosity on the addition of acids, subtracting the initial value from the final value and plotting this difference gave relatively large values for the constant *b*.

The values for the constant *b* are higher for the series with the electrolytes present than for the series with the electrolytes removed. The wires used in the two series both gave a reading of 29.2° M. with a 60 per cent sucrose solution whose absolute viscosity is 43.86 centipoise at 25° C., the temperature at which all observations were made. Unfortunately, the wire of the viscosimeter broke after one series had been completed and a new wire was used for the other series. The breaking of the wire was a great misfortune, because it leaves the question of the effect of the salts present in the original flour, on the slope of the curve, still unanswered. The results do show, however, that the salts present in the flour exert an effect which differs in different flours, indicating that the electrolytes present in the various flours are different. This is also shown in Figures 3 and 4 and may be the explanation for the different order of the acids when arranged in the order of their power to produce maximum imbibition. The order of the acids was found to be more nearly the same when the electrolytes were removed. See Figures 6 and 7. The effect of the actual quantity of supposedly the same electrolytes from the same wheat is shown in a comparison of the patent and clear flours from the same wheat in Table XXVIII.

TABLE XXVIII

Relation Between Patent and Clear Flours Milled from the Same Wheat and the Constant *b* of the Logarithmic Equation

Lab. No.	Constant *b*, electrolytes present	Constant *b*, electrolytes removed	Flour grade
1001	3.705	3.023	Patent
1002	2.689	2.926	First clear
1005	2.766	2.666	Patent
1004	2.829	2.552	Second clear
1006	2.996	2.610	Patent
1007	2.829	2.568	First clear

The greatest deviation between the constants *b* is shown when the electrolytes were present. Patent flour 1001 gave a constant of 3.705 and clear flour from the same wheat, 1002, gave a constant of 2.689. In the other two cases the agreement is better. When the electrolytes were removed, the patent flour in each case gave a slightly higher constant than the clear flour, indicating that the protein in the

patent flour was of only slightly better quality than the protein in the clear flour. This agreement is also an indication that the influence of the electrolytes present in the flour was largely eliminated, so far as their effect on the evaluation of the imbibitional properties of the gluten was concerned, by the method of leaching used for their removal.

The constant K, obtained by dividing the product of the glutenin concentration and the constant *b*, by the loaf volume, shows considerable fluctuation in the series with the electrolytes present. When the electrolytes are removed we find the constants apparently fall into three groups. With the exception of flour 1010, the first group contains patent or straight flours which would be used for bread making; the second group contains flours which if used by themselves would be considered pastry flours and would be used commercially for bread making only when blended with other flours of considerable baking strength; the third group contains the clear flours. The average of the constants K in the first group is 0.00829, the average deviation from this value is 1.99 per cent, and the maximum deviation is 4.1 per cent. The average in the second group is 0.00661, while the average and maximum deviation is 3.93 per cent. The average in the third group is 0.01089, the average deviation is 2.91 per cent, and the maximum deviation is 4.41 per cent. The greatest experimental error is believed to be in the determination of the glutenin content of the flours. These results indicate a rather definite relation between the glutenin content, the increased concentration effect of imbibition produced by lactic acid, and the loaf volume. It is generally accepted that the actual volume of the loaf obtained depends on many factors, and it could hardly be expected that the measurement of one factor would determine the loaf volume. These results indicate that within each group there is a definite relation between the glutenin content, the quality content *b*, and the loaf volume. The groups of different constants are probably produced by other factors. Just what these other factors are has not been determined, other than the relation expressed in Table XXVII between the class and grade of wheat and the grade of flour. Some correlations which might be drawn from the work carried out by Rumsey (1922) and Collatz (1922) using these same flours might be mentioned here, more in the way of discussion than of definite conclusions. Collatz found that by the addition of malt flour or extracts the loaf volume of flours 1002 and 1007 could be increased about 16 per cent. Increasing the loaf volumes used for these flours in Table XXVII by 16 per cent we obtain the constant 0.00833 for flour 1002 and 0.00922 for flour 1007. The volume of flour 1001 was increased 10 per cent, which gives a constant of 0.00779 instead of 0.00857. Flour 1008 showed no increase in volume

on the addition of malt flour or extract. Flour 1003 showed an increase of 12 per cent which changes the constant to 0.00566. It is undoubtedly unfair to take the increase of this flour as 12 per cent, for this great increase occurred with only one loaf and was apparently too high. Rumsey (1922) has shown that flour 1004 has about the same diastatic power as flours 1002 and 1007. It is probable that an increase in volume might have been obtained with this flour by the addition of diastase. Table XXIX gives the relative diastatic powers of the flours as determined by Rumsey. The hydrogen-ion concentration of the flour and the fermented dough at the time of going into the oven are taken from the paper by Collatz (1922).

TABLE XXIX

Maltose Produced in One Hour by 10 Grams of Flour in 100 cc. of Water at 27°C.; the pH Value of a 1 to 5 Flour-in-Water Suspension, and of the Fermented Dough at Time of Going Into the Oven

Lab. No.	Maltose produced, mgms.*	Flour, pH†	Fermented dough, pH†
1009	211.8	....	....
1001	248.2	5.81	4.79
1008	304.1	5.98	5.19(?)
1005	131.9	5.84	5.17
1010	92.6	....	....
1006	105.7	5.78	5.03
1011	51.7	6.15	4.92
1003	34.8	6.00	5.05
1002	186.5	6.05	5.05
1007	123.6	6.10	5.53
1004	145.0	6.17	5.80

* Determinations by Rumsey (1922).
† Determinations by Collatz (1922).

Table XXIX shows that only two flours, namely, the clear flours 1004 and 1007, did not reach approximately a pH of 5.0, which the work of Jessen-Hansen (1911) indicates to be the general optimum for bread making. Thus, one reason for the low volumes of these two flours may have been that the optimum hydrogen-ion concentration had not been reached. The clear flour 1002 did reach this optimum, and making the correction for the increased volume produced by added diastase yields a constant which agrees with those in Group 1. This indicates that the factor causing the low volume was insufficient diastase and not the buffer effect due to grade. Flour 1007 was improved by added diastase, yet it still gives a constant which is too high for Group 1. It may be that the low loaf volume in this case is caused by two factors, namely, the low diastase content and the failure of the dough to reach the optimum hydrogen-ion concentration for bread baking, owing to its high buffer action.

If we take the first group of flours for comparison, then the second group yields larger loaf volumes than their glutenin content and its imbibitional properties would indicate. No hypothesis will be expressed as to the factors operative in the formation of Group 2. The flours in Group 2 are especially low in diastase. An increase in diastatic power would have a tendency to increase the loaf volume and yield a still lower constant.

While eleven flours with such meager data are far too few upon which to base definite conclusions, this work indicates that the glutenin content and its imbibitional properties may be used as a measure of one of the main factors concerned with baking strength. While the data presented here indicate three groups of flours as shown by the imbibitional studies, later work may show more than three groups. In the method used, all the factors which go to make up viscosity and plasticity were probably operative.

Gröh and Friedl (1914) found that the gliadins in wheat and rye flour were identical in physical properties. Lüers and Ostwald (1919) found that the viscosity of wheat flour suspensions was increased by the addition of lactic acid, while the viscosity of rye flour suspensions remained unaffected. The experiments reported here indicate that the colloidal properties of glutenin, as shown by its imbibitional power as affected by acids and alkalies, constitute one of the factors concerned in strength of flour. A comparison of the results reported here with those of Lüers and Ostwald would indicate that the glutenin of rye flour is markedly different from the glutenin of wheat flour; and from the results of the studies on the viscosity of wheat gluten reported here, it would be expected that rye flour would behave as an extremely weak flour.

Guthrie (1896) seems to be the first investigator who definitely attributes the strength of flour to the glutenin. Since the experiments reported in the present paper were completed, Woodman (1922) has reached the conclusion that the glutenin fraction is concerned in the problem of flour strength. Woodman extracted the proteins, gliadin and glutenin, from samples of strong (Canadian) and weak (English) flours and then followed the changes in optical rotation of these pure proteins when they were racemized with dilute alkali at 37°. He concludes that the gliadin fractions of both strong and weak flours are identical in physico-chemical behavior but that there is a marked difference in the glutenins. He states, "The glutenines from the two types of flour have been shown to be two distinct substances, this conclusion being based on their different optical behavior during racemization by dilute alkali.

"It is suggested that the existing ideas on flour strength require modification to include the facts recorded in this investigation. It is desirable to retain the dual conception of strength as put forward by Wood. The factor which determines the size of the loaf is most probably connected with the diastatic capacity of the flour, as was suggested by this investigator. On the other hand, the factor which determines the shape of the loaf and which appears to be directly related to the physical properties of the gluten of the flour, is possibly dependent on the particular glutenine mechanism possessed by the wheat.

"The results of this investigation suggest that the strong wheat synthesizes one type of glutenine and the weak wheat a different type, whilst wheats of intermediate strength may contain varying proportions of the two glutenines."

It will be noted that these conclusions are very similar to our own, reached from an entirely different sort of evidence, and we regard Woodman's work as strongly confirming the hypothesis that flour quality is intimately associated with the glutenin fraction of the wheat proteins.

While the present investigation indicates that the amount and colloidal properties of the glutenin are important factors concerned with flour strength, more extensive investigations must be carried out before final conclusions can be drawn.

NATURE OF VISCOSITY CHANGES INDUCED BY ADDITION OF ACIDS AND ALKALIES

The viscosity curves shown in Figures 3, 4, 6, 7, 8, and 9 of the present paper are very similar in appearance to those which Loeb (1920a, 1920b, 1920c, 1921a, 1921b, 1921c, 1921d, 1921e, 1921f, and 1922) presents for gelatin, casein, etc. Loeb interprets his results as being due to stoichiometrical combination of the protein with the acids or bases followed by ionization of the protein salt and hydration of the protein-ion by osmotic phenomena governed by the Donnan (1911) equilibrium. In other words, Loeb apparently takes the view that imbibition changes in a protein system are due to chemical reactions, whereas the prevalent viewpoint of workers in the field of colloids is that they are due to surface phenomena. Certain of the experiments which we have reported appear to throw light upon these questions.

The experiments reported in Tables XIX and XX show that gliadin is relatively soluble in distilled water and that the gliadin can be almost completely removed from wheat flour by leaching with water. It will be noted from Table XV that 18 grams of flour 1009, from which the soluble salts had been removed by leaching with one liter of water

followed by 500 cc. of water, attained a maximum viscosity of 258° M. when treated with lactic acid.

As shown in Table XIX, the same treatment followed by four extractions with alcohol gave viscosity readings of 191° M. and 195°M.; and (Table XX) sixteen extractions with 500 cc. distilled water followed by an extraction with alcohol gave a viscosity reading of 149° M.

It might be argued from the above experiments that the alcohol removed some of the protein, which was responsible for the increase in viscosity produced by the addition of lactic acid. That this is not the case, however, is shown by Table XXI and Figure 12. In this instance 18 grams of the same flour was leached with from 8 to 16 successive portions of 500 cc. each of distilled water, *but the alcohol extraction was omitted* and a maximum viscosity of 456° M. was attained. Apparently the alcohol treatment was responsible for the lower viscosities shown in Tables XIX and XX.

This enormous increase in viscosity from 4° M. to 456° M. was produced by the addition of 0.4 cc. of a normal solution of lactic acid, and an increase in viscosity from 4° M. to 365° M. was caused by even 0.10 cc. of normal lactic acid.

According to Loeb's hypothesis, in order that viscosity changes may take place, there must first be a chemical combination of the acid with the protein, followed by the ionization of the protein salt and the subsequent hydration of the protein ion through osmotic behavior controlled by the Donnan equilibrium. It appears very improbable that equilibrium would be reached practically instantaneously if osmotic phenomena are involved; nevertheless, equilibrium was reached practically instantaneously (within 2 or 3 seconds) in our experiments. On the addition of 0.1 cc. of normal magnesium sulfate solution to such a system, the viscosity fell practically instantaneously to a small fraction of its former value. Here again the rapidity of the changes is not suggestive of osmotic behavior. It appears far more probable that the reactions involved are due to surface phenomena, and the viscosity changes on the addition of acid and salt may be likened to the changes which take place when an electric current is passed through an electro-magnet suspended above a pile of iron filings and the current is later broken by throwing the switch. Apparently on the addition of the lactic acid a comparatively thick "shell" of water is bound upon the surface of the glutenin particle, only to be instantly released when the salt is added. If the water penetrated into the interior of the glutenin particle by osmosis, there should be a gradual fall in viscosity when dehydration takes place. It has been impossible to demonstrate such a slow fall, equilibrium being attained practically instantaneously.

Again, if the view is correct that hydration of proteins is a phenomenon controlled only by the hydrogen-ion concentration, it is impossible to understand why glutenin shows such marked imbibition changes in contrast to the behavior of the prolamine, gliadin, or why glutenin from a strong flour shows such marked differences in behavior from glutenin from a weak flour *at the same hydrogen-ion concentration and salt content.* In our experiments, hydrogen-ion concentration does markedly influence imbibition; nevertheless, the fact remains that "there is an inherent difference in the glutens from strong and weak flours. The physico-chemical properties of the glutens from the different flours are not identical and would not be identical even if the flour had originally had the same acid (hydrogen-ion concentration) and salt content. The difference between a strong and a weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which these properties are much less marked." These statements were made by Gortner and Doherty in 1918, and our present work seems to emphasize their findings. We have advanced the knowledge of the flour strength problem so as to limit, apparently, the difference of colloidal behavior to the glutenin fraction of the flour proteins, but the difference in the glutenin is apparently due to its behavior as emulsoid colloids. This is particularly apparent in the baking tests (Table XXIV, Plates 1 and 2) in which an alteration in the colloidal properties of the glutenin ruined the baking value of the flour. Other experiments which are now in progress point to the same conclusions.

SUMMARY AND CONCLUSIONS

Viscosity of flour-in-water suspensions was used as an easy, rapid, and accurate method for the investigation of the comparative imbibitional properties of the flour proteins. Viscosity studies were made on a series of wheat flours of various grades and baking strength in an effort to investigate the relation of the colloidal properties of wheat proteins to the strength of flour.

Suspensions of flour in water are extremely labile systems and are affected by the many factors which influence the viscosity of emulsoid colloids.

Maximum viscosity produced by the various acids acting on wheat flours occurs at approximately the same hydrogen-ion concentration regardless of the acid. The hydrogen-ion concentration for maximum viscosity was found to be slightly higher than a pH of 3. The highest viscosity produced by the different acids occurs at practically the same hydrogen-ion concentration with the different flours.

Different acids apparently do not produce the same maximum imbibitional effect with wheat proteins.

Materials extracted from flour by water influence the imbibitional power of the various acids in different degrees. The water extract from various flours differs in its effect on imbibition produced by different acids. The series of acids arranged in the order of their ability to produce maximum viscosity is more nearly the same for the different flours after the removal of the soluble extract.

Salts added to the flour-in-water suspension apparently divide themselves into sharply defined groups in respect to their ability to inhibit imbibition produced by lactic acid. These groups were found to be the same with the two flours investigated.

Failure of some acids to produce imbibition is probably related to their effect as protein precipitants.

Viscosity in the presence of alkalies is not so markedly affected by the soluble materials present in the natural flour as in the presence of acids. The maximum viscosity produced by alkalies occurs at approximately a pH of 11.

Maximum viscosity and concentration of flour were found to conform to the logarithmic expression,

$$\text{Log. of viscosity} = a + b \text{ (log. of concentration)}$$

in which the log. of viscosity is the logarithm of the maximum viscosity obtained with lactic acid.

Repeated extraction of flour with distilled water removes practically all the alcohol-soluble proteins. The first extraction with water removes proportionately more than the subsequent extractions.

Glutenin is the protein mainly responsible for the marked imbibitional power of flour and gluten as influenced by acids and alkalies. The treatment of glutenin of flour with alcohol of a concentration of 70 per cent or more, markedly affects the colloidal properties of the glutenin as measured by viscosity. The imbibitional strength of the glutenin is probably represented most accurately by the constant b of the logarithmic expression noted above.

These results seem to indicate that the glutenin content and its imbibitional properties should be taken into account in investigations of flour strength.

Doughs which have been brought to a pH of 3.0 or 11.0 by the addition of acid or alkali and then the acid or alkali neutralized, have lost their baking strength.

Flours that contain a protein which responds markedly to the increasing imbibitional effect of lactic acid lose their baking strength if they are doughed up with 70 per cent or 95 per cent alcohol and

then dried. The fact that the baking strength of flour is lost by extraction of the flour with alcohol is not proof that the gliadin is the protein mainly concerned with flour strength.

The viscosity determinations indicate that the effect of the alcohol is due mainly to changes produced in the colloidal properties of the glutenin.

The earlier conclusion of Gortner and Doherty, that there is an inherent difference in the physico-chemical properties of the gluten from strong and weak flours and that these differences are due to the colloidal state of the gluten proteins, is confirmed. In addition, it has been shown that the differences in colloidal properties apparently reside in the protein, glutenin.

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BIOGRAPHY

Paul Francis Sharp was born December 6, 1894, at Allen, Nebraska. He attended the public schools of Randolph, Walthill, Lincoln, and University Place, Nebraska, and the high schools of University Place and York, Nebraska, graduating from York high school in the spring of 1914. Entering Nebraska Wesleyan University in the fall of 1914, he specialized in chemistry and graduated with the A.B. degree in June, 1917. Early in the fall of 1917 he entered the army and after three months at Camp Funston, Kansas, was transferred to Ordinance Department and assigned to Chemical Warfare work at the American University, Washington, D. C. He was later transferred to the Chemical Warfare Service. His work at the American University was in the Pharmacological Research Section under the direction of Dr. E. K. Marshall, Jr. He was discharged from the army in December, 1918, and in January he entered upon his graduate work at the University of Minnesota, majoring in the Division of Agricultural Biochemistry. He held the position of research assistant in agricultural biochemistry through the spring and summer of 1919 and in the fall of 1919 was made an instructor in the department, which position he held until the summer of 1922.

In June, 1920, the degree of Master of Science was conferred upon him by the University of Minnesota, his thesis subject being "Some Physico-Chemical Properties of the Glutens from Strong and Weak Flours."

In May, 1922, he presented to the faculty of the graduate school a thesis on, "Viscosity as a Measure of Hydration Capacity of Wheat Flour and Its Relation to Baking Strength" in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The degree was conferred June 14, 1922, by the University of Minnesota.

Member of the American Chemical Society, American Association for the Advancement of Science, Gamma Alpha, Phi Lambda Upsilon, Sigma Xi, and Gamma Sigma Delta.